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Further Investigations Concerning the Stimulating Effect of Anterior Pituitary Gland Preparation on the Thyroid Gland.

LEO LOEB, R. B. BASSETT AND HILDA FRIEDMAN.

From the Department of Pathology, Washington University School of Medicine.

We have shown that while feeding of Armour's tablets of anterior pituitary has an inhibiting effect on the activity of the thyroid gland as indicated by the structural changes it induces,[1] injections of acid or alkaline extracts of anterior pituitary prepared in our own laboratory from cattle gland have a very markedly stimulating effect, comparable to that characteristic of the thyroid in typical Graves' disease.[2] We furthermore compared with the effects of the extracts, the effects of inoculation of anterior pituitary gland substance as such, obtained from guinea pig, rabbit, or cattle. These investigations suggested the conclusion that there are apparently several active substances in the anterior pituitary and that the substance responsible for the growth of the ovarian follicle and ovulation is not identical with the substance responsible for the hypertrophy of the thyroid gland, but that the latter seems to be identical with the substance which causes the production of interstitial gland and of pseudolutein bodies in the ovary of the guinea pig.[3] Silberberg[4] has

[1] Loeb, Leo, *Am. J. Path.*, 1926, **2**; 1920, **42**, 77. Loeb, Leo, and Kaplan, E. E., *J. Med. Res.*, 1924, **44**, 557.

[2] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

[3] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.

[4] Silberberg, Martin, PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 166. *Krankheitsforsch.*, 1930, **8**, 171.

shown that if acid extract of anterior pituitary and KI, both of which given singly stimulate the thyroid gland, are administered simultaneously, not only is there a lack of summation of the stimulating effects exerted by these substances, but a diminution of the hypertrophy which otherwise would have been caused by acid extract of anterior pituitary alone. We now report on the continuation of our investigations into the relation between the anterior pituitary and thyroid glands.

I. *Effect of administration of thyroid substance on the hypertrophy caused by acid extract of anterior pituitary.* Loeb[5] found that daily administration of 0.1 grain tablets of thyroid substance (Armour's) to guinea pigs in which the greater part of the thyroid had been removed, prevented the occurrence of hypertrophy, whereas administration of KI did not have this effect. Similarly simultaneous administration of thyroid substance was able to prevent almost entirely the increase in activity in the thyroid gland caused by feeding of potassium iodide (Gray and Rabinovitch).[6]

In the case of the hypertrophy produced by injections of acid extract of anterior pituitary, Silberberg, as stated above, found that feeding with KI causes a diminution in the hypertrophy. In the following experiments begun in December, 1929, we studied the effect of feeding of thyroid substance on the hypertrophy ordinarily caused by acid extract.

We used, in a large majority of cases, guinea pigs weighing between 170 and 200 gm. Altogether 36 experiments were carried out. In 19 experiments we gave the combined treatment, in 17 control guinea pigs, either anterior pituitary extract—1 cc. of the extract, prepared as stated previously,[2] was injected daily—or thyroid tablets each containing 0.1 grain thyroid substance were administered singly. In some of the 19 experiments, both substances were administered concomitantly daily over a period of 15 days. In other experiments, in a preparatory period lasting 3 to 5 days, the thyroid tablets alone were given each day, while in a succeeding period, varying in length between 3 and 8 days, both thyroid tablets and pituitary extracts were given combined; the preparatory period was inserted in order to increase the chance for the thyroid substance to produce its effect. For the same purpose, in a number of experiments we increased the quantity of thyroid substance from one tablet to 2 tablets daily. In the former experiments to which we referred only one tablet had been given. Notwithstanding these measures

[5] Loeb, Leo, *J. Med. Res.*. 1920, **41**, 481.

[6] Gray, S. H., and Rabinovitch, J., *Am. J. Path.*, 1929, **5**, 485.

tending to intensify the thyroid effect, we found that thyroid substance could only to a certain extent diminish, but was not able to prevent the hypertrophy caused by extracts of anterior pituitary substance. As a result of the concomitant action of thyroid substance, the colloid was usually present in a larger quantity, remained somewhat harder and did not undergo solution processes and absorption with the same intensity as in experiments in which acid anterior pituitary extract alone was given; also with the combined treatment the mitoses were less numerous and the acinus cells a little lower. On the other hand, in some cases there was hardly any effect of the administration of thyroid substance. There was, therefore, some variation present in the intensity of the action of thyroid substance, but in general the latter caused merely a certain diminution in the intensity of the hypertrophy without preventing it. Thus, the administration of thyroid substance was much less potent in mitigating the effects of anterior pituitary extract than in diminishing compensatory hypertrophy or the stimulating effects of KI. It was possible to prevent the latter 2 processes almost entirely through feeding of thyroid tablets, while as stated, the effects of extracts of anterior pituitary could at best be made less intense. We have in all these cases to deal with quantitatively balancing conditions. These observations prove that the injection of extracts of anterior pituitary is much more potent in producing hypertrophy of the thyroid gland than removal of a great part of the gland or administration of stimulating doses of KI.

This result is of interest also in another respect. The fact that the administration of thyroid substance prevents compensatory hypertrophy might be intepreted as indicating that lack of thyroid hormone directly or indirectly acts as a stimulant on the thyroid gland, and that if we supply the necessary amount of hormone by feeding thyroid, this stimulus is lacking and therefore compensatory hypertrophy does not take place. However, the fact that thyroid substance also inhibits to some extent the effect of substances directly stimulating the thyroid epithelium, indicates that the presence of thyroid hormone or of a substance associated with it exerts a continuous depressing effect on the activity of the thyroid gland and in addition renders it less responsive to various kinds of stimulation. Furthermore, it suggests the possibility that the greater the amount of thyroid hormone which is available in the organism, the lower is the level of thyroid activity and responsiveness. We intend to test this suggestion in further experiments.

II. It was of interest to determine the time necessary for the

return of the thyroid gland to its normal condition after hypertrophy had previously been produced through administration of acid extract of anterior pituitary gland, and also to compare the rate of retrogression subsequent to the cessation of the injections of the extract with the rate of retrogression observed by Rabinovitch[7] after cessation of the administration of potassium iodide. One cc. of acid extract of anterior pituitary was injected daily for 5 consecutive days. The injections were then discontinued and the gland allowed to recover.

Briefly the results were as follows: One day after completion of the series of injections, we found a very marked hypertrophy. Numerous mitoses were present and a great part of the colloid had been absorbed. The shape of the acini was usually irregular and in many acini the lumina had been reduced to slits. After 3 days there was still a marked hypertrophy, but it was not quite so great as before; in some places the epithelium was less high. New solid colloid began to be produced at this period and at the same time the number of mitoses was much reduced. Further retrogression was noted after 6 days, when the harder colloid was still more increased in quantity. Again we found the number of mitoses very much reduced. Yet, while these changes had taken place, on the whole, there was much hypertrophy noticeable. After 9 days moderate hypertrophy was observed in some acini, but in other areas the hypertrophy was very much diminished. The acini generally contained solid colloid, although in a number of acini peripheral vacuoles were present in the colloid. After 11 days there was still slight hypertrophy in some places. After 22 days the thyroid had almost become normal; yet the epithelium was slightly higher than in a normal gland. In addition some irregular acini were found. After 31 days the hypertrophy had disappeared and the gland had regained its usual appearance.

Comparing with the results obtained by Rabinovitch in the case of hyperactivity of the thyroid gland caused by KI it will be noted that in the latter case the return to a normal state was in certain respects similar to that observed after anterior pituitary extract administration. In both cases the mitoses fell very suddenly within a few days after cessation of stimulation; likewise in both series of experiments hard colloid was soon secreted and it took the place of the softened colloid. However, while after cessation of KI feeding the hypertrophy disappeared within 2 weeks, a certain degree of hypertrophy following injection of pituitary extracts remained visi-

[7] Rabinovitch, Jacob, *Am. J. Path.*, 1930, **6**, 71.

ble up to at least 3 weeks. This delay in retrogression corresponds to the greater intensity of the changes produced by acid extract of anterior pituitary as compared with the changes produced by potassium iodide administration.

III. While all the doses of acid extract of anterior pituitary used by us produced marked hypertrophy, and while in some cases smaller doses could produce about as marked hypertrophy as the larger ones, in general, it was found that the hypertrophic changes caused by daily injection of 3 cc. of the extract were greater than those caused by daily injections of 1 cc. and ½ cc. of the extract. Thus, after a daily injection of ½ cc. of acid extract during a period of 6 days, marked hypertrophy of the thyroid was obtained. Instead of typical round or oval acini we found some slits in the center of the glands; in other acini peripheral vacuolization in the colloid was noticeable. Mitoses could be seen in increased number and the epithelium was high. Yet there was more colloid retained in the acini than after daily injection of 3 cc. extract over the same period of time, and some of the colloid was relatively hard and slightly retracted. However, even after daily administration of 3 cc. acid extract, the peripheral acini are still relatively wider and contain a larger amount of colloid than the central ones; but in these glands the colloid which is still present in the peripheral acini, is usually very soft, or it may disintegrate to small granules. While there is thus no direct correspondence between the amount of acid extract injected and the intensity of hypertrophy attained, on the average, larger doses of acid extract of anterior pituitary are more effective than smaller ones.

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Cranial and Cranio-Tympanic Bone Transmission.

A. G. POHLMAN.

From the St. Louis University School of Medicine.

The perceptive mechanism of the internal ear may be said to occupy a position of reasonable physiological quiet so far as air sounds are concerned because the end organ is immersed in liquid and because of the depth to which the apparatus is buried away from the surface. The writer has shown elsewhere[1] that this acoustic insula-

[1] Pohlman, A. G., *Anat. Rec.*, 1930, **45**, 236.

tion amounts to about 30 sensation units or a factor of 1000 times the normal threshold intensity. This indicates the functional capacity of the sound transmission apparatus which may be likened to a matching transformer with the larger area at the low impedance end of the system (drum membrane) and the smaller area (stapes footplate) at the high impedance end.

If, however, the vibrations are transmitted directly to the cranial bones through the stem of a fork or with a bone telephone, then a somewhat different physiological condition obtains. The vibrations necessarily pass through all structures including the cochlea and the sound transmission system. The evidence indicates that the activating factor under these conditions is a direct transmission of the vibrations through the wall of the otic capsule (cranial transmission). This means that the vibrational thrusts through the air sound transmission apparatus do not contribute enough increased intensity to that already developed within the cochlea by direct transmission to come to consciousness. The result is that bone acuity is not influenced by the functional condition of the air sound transmission system proper. This point is of considerable diagnostic significance.

When the external auditory canal is occluded, or when the drum membrane has been loaded with liquid, the mechanical conditions for the transmission of both air and bone sounds are changed. The air acuity is more markedly lowered at the high frequency end and the normal individual presents a curve which is quite similar to a typical internal ear disability. The differences in the efficiency for the transmission of lower and higher frequencies is undoubtedly related to the weight of the occluding or loading factor because the larger the mass the relatively less well it responds to high frequencies. When the bone acuity is tested under the same conditions one finds the high frequency end of the audible field unaffected, while the low frequency end shows an enhanced acuity which is known as prolonged bone transmission by the otologists. The suggested explanation is that at the high frequency end the vibrations of the plug or liquid are too poorly developed to contribute to the intensity already registering in the cochlea but at the low frequency end the vibratory responses through the air sound transmission apparatus are sufficiently great to bring this route into prominence. The reason why no definite separation between this abnormal cranio-tympanic and the normal cranial bone transmission has been made is because of the limited range of the fork tests, and audiometric methods with a bone telephone were required to establish it. The abnormal cranio-

tympanic bone transmission occurs in any case with a functional air sound transmission system where the external auditory canal is occluded or where the drum membrane is loaded with liquid either on its lateral or on its deep surface. It does not take place when the air sound transmission apparatus is functionally out of commission or has been removed.

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A Quantitative Test for the Determination of Xanthine and Hypoxanthine in the Blood.

WARREN H. COLE, WILLIAM H. ELLETT AND NATHAN A. WOMACK.

From the Department of Surgery, Washington University School of Medicine and Barnes Hospital, St. Louis, Missouri.

Working on the basis of the assumed fact that the enzyme, xanthine-oxidase, is specific for the conversion of xanthine and hypoxanthine to uric acid, we have devised a test for these 2 compounds in the blood. The enzyme is prepared from fresh milk after the method of Dixon and Thurlow.[1] A small amount of enzyme (50 mg.) is placed in a test tube containing 2 cc. of oxalated blood and incubated for 24 hours along with a control tube containing 2 cc. of oxalated blood from the same patient. After incubation for 24 hours at 37°C., a uric acid determination (Benedict Method) is made on the contents of each of the tubes. The difference in the amount of uric acid found in each of the tubes is presumably explained by the conversion of the xanthine and hypoxanthine of the blood by the xanthine-oxidase added to the blood in one of the tubes. We were surprised to learn that the increase in the amount of uric acid was quite routinely about 200% in the series of normal individuals upon which the test was performed. Up to date, however, no constant variation has been found in any of the group of diseases to which we applied the test. As far as we know the only other test available for the determination of xanthine and hypoxanthine in the blood is the nephelometric method devised by Graves and Kober in 1915.[2] They have apparently not applied their test to human blood, however.

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1 Dixon, M., and Thurlow, S., *Biochem. J.*, 1924, **19**, 971.

2 Graves, S. S., and Kober, P. A., *J. Am. Chem. Soc.*, 1915, **37**, 2430.

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Some Physiological Properties of a New Tri-Atomic Alcohol from the Urine of Pregnant Women.

EDWARD A. DOISY, J. M. CURTIS, L. LEVIN, P. A. KATZMAN AND S. A. THAYER.

From the Laboratory of Biological Chemistry, St. Louis University School of Medicine.

A study of the responses of female mice, rats, guinea pigs and rabbits to the enteral and subcutaneous administration of the new triol[1] has given interesting data. Using rats 17-23 days of age it was found that minute quantities administered either subcutaneously or by stomach tube caused opening of the vagina in from 2 to 10 days. Vaginal smears made twice daily showed that cornification generally began on the third day following; the cornified cells persisted from 2 to 5 days. Animals sacrificed at the time leucocytes began to appear showed either large follicles or corpora lutea and sometimes both. That the new triol acts in the absence of the ovary is proved by the administration to ovariectomized rats 21-22 days of age. Opening of the vagina occurred in 2 to 7 days with the subsequent appearance of cornified cells in the smears.

Although our experiments are still incomplete our evidence indicates that if the amount required to produce opening of the vagina of 21 day old animals be regarded as a unit, the rat unit weighs 0.10γ or less and the mouse unit 0.004γ (1γ = 0.001 mg.).

Dr. W. D. Collier is making microscopic studies of our injected animals.

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Effect of Glucosamine and of Glycollic Acid on Detoxication of Sodium Benzoate in Rats.

WENDELL H. GRIFFITH.

From the Department of Biological Chemistry, St. Louis University School of Medicine.

Survival and growth of young rats on diets containing sodium benzoate are possible only if the diets furnish a supply of glycine, or of a precursor, adequate for the detoxication of the benzoate and for

[1] Doisy, E. A., Thayer, S. A., Levin, L., Curtis, J. M., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 88.

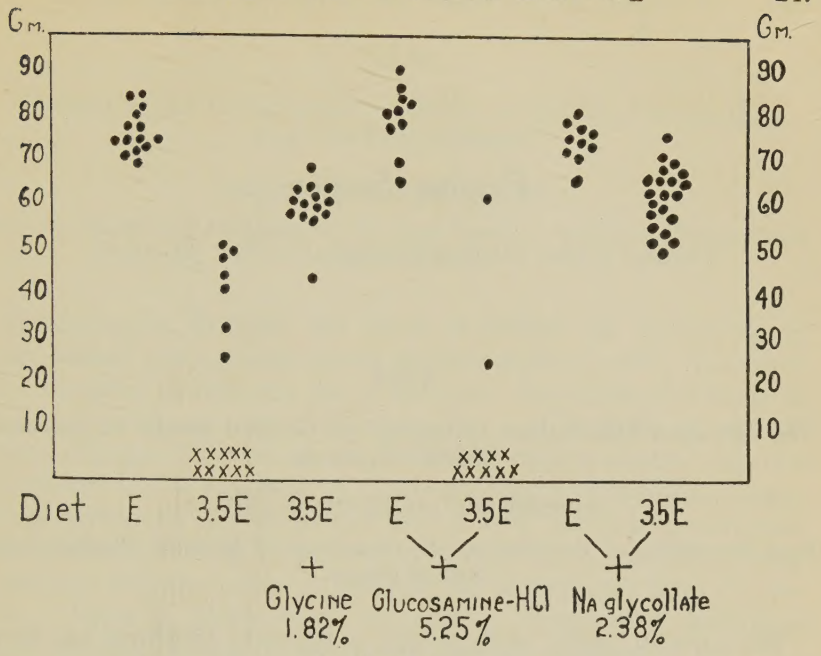

CHART 1.

Increase in weight of young male rats during a 40-day experimental period on diets containing sodium benzoate. The per cent of sodium benzoate added to the basal diet, E, is indicated by the number preceding the letter E. The food intake was restricted, each rat receiving the same quantity of food of equivalent calorific value (975 calories). Rats were kept in raised cages. Deaths during the experimental period are represented by X.

the formation of new tissue proteins. Previous experiments have demonstrated that the injurious effects of toxic benzoate diets disappear if glycine is added to the diets.[1] On the basis that readily available precursors of glycine might show a protective action similar to that of glycine, a search is being made for such precursors. Results of experiments with glucosamine and glycollic acid are reported in this paper.

Quantities of these compounds, equivalent to the benzoate concentration in the experimental diets, were found to be non-toxic when added to the basal diet. (Chart 1.) No change in the degree of survival or in the rate of growth resulted from the addition of neutralized glucosamine hydrochloride to the toxic benzoate diet. On the other hand, the addition of sodium glycollate to the toxic benzoate diet afforded practically the same protection as that furnished by glycine itself. These experiments are being continued in order to determine whether glycollic acid is actually a precursor of glycine in the animal body.

[1] Griffith, W. H., *J. Biol. Chem.*, 1930, **85**, 751.

Peiping Section.

Peiping Union Medical College, October 30, 1930.

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The Nitrogen Metabolism in Infants on Graded Intake of Soybean "Milk" Proteins.

ERNEST TSO AND FU-T'ANG CHU.

From the Division of Pediatrics of the Department of Medicine, Peiping Union Medical College.

The soybean "milk" with certain non-protein additions has been successfully employed in the feeding of young infants.[1] In general the proteins are fed on a high level constituting approximately 20% of the caloric intake in contrast to the usual 12 to 15% in case of cow's milk and 8% human milk proteins. The nitrogen metabolism was followed in 2 male infants 4 and 7 months old who were given soybean "milk" formulae furnishing approximately 18.5, 13.5, 10.5 and 8.5% protein calories respectively but the same number of total calories per kilo of body weight. In one period cow's milk protein was fed at 12.2% level of calorie intake. The results were:

1. At each of the graded levels of protein intake the nitrogen balance was positive. But the higher the protein intake the larger was its retention.

2. Retention was better on 12.2% calorie intake in cow's milk proteins than on 18.5% of soybean "milk" protein calories.

3. Within the limits studied, the ratio of soybean "milk" nitrogen absorbed to the nitrogen ingested seems fairly constant. The soybean nitrogen absorption was distinctly lower than in the case of cow's milk nitrogen.

[1] Tso, E., *Chinese J. Physiol.*, 1928, **2**, 33. *Am. J. Physiol.*, 1929, **90**, 542. *Nat. Med. J. China*, in press.

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Changes in the Composition of Blood in Rabbits Fed on Raw and Cooked Soybeans.

ERNEST TSO AND S. M. LING.

From the Division of Pediatrics of the Department of Medicine, Peiping Union Medical College.

According to Horvath[1] the blood of rabbits fed exclusively on water-soaked raw soybeans shows an increase in uric acid, urea nitrogen, inorganic phosphorus and cholesterol. Extending this study to cooked soybeans we obtain these results: 1. There are no demonstrable changes in the blood composition of rabbits whether they are fed cooked or raw soybeans or the control diet of millet and cabbage except perhaps in cholesterol content. 2. Uric acid is present in rabbit's blood only in negligible quantity. 3. The blood cholesterol value is perhaps slightly higher in animals fed on soybeans than in controls. There is, however, no appreciable difference in cholesterol levels between rabbits fed on cooked and raw soybeans.

The experimental data are summarized in Tables I and II. It should be noted that samples of blood were obtained not from the marginal ear vein but heart puncture. Both Folin and Benedict's methods failed to show any measurable quantity of uric acid in rabbit's blood. Only by employing Folin's improved method[2] and at the same time increasing the blood filtrate from 5 to 10 cc. and reducing the standard to 2 cc., the uric acid figures given in Table I were obtained.

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[1] Horvath, A. A., *J. Biol. Chem.*, 1926, **68**, 343.

[2] Folin, O., *J. Biol. Chem.*, 1930, **86**, 179.

TABLE I.

Date	Rabbit No.	Weight	Food	Uric acid mg. %	Urea N mg. %	Inorganic P mg. %	Cholesterol mg. %
April 10	1 ♂	1550	Millet	Faint trace	20.4	3.50	77.0
	2 ♂	1540	and	" "	26.6	3.35	67.7
	3 ♀	1640	cabbage	" "	23.8	4.73	61.5
	4 ♀	1380		" "	26.6	4.87	84.0
	6 ♂	1560		" "	21.0	3.65	85.1
	7 ♀	1550		" "	22.4	3.44	63.4
	8 ♀	1900		" "	26.6	3.06	58.0
April 11	9 ♂	1500	Millet	0.85			
	10 ♀	1400	and	0.86			
	11 ♀	1570	cabbage	0.95			
	13 ♂	1340		0.84*			
	14 ♀	1880		0.89			
	15 ♀	1800		0.93			
	16 ♀	1600		0.85			
April 23	3	1665	Raw	1.01	23.0	5.0	90.9
	6	1550	soybeans	0.89	20.7	4.2	78.4
	7	1930	(2 weeks)	0.83*	21.7	3.9	83.3
	8	1860		0.93	25.9	3.4	81.2
	9	1570	Cooked	0.92	24.9	3.9	77.0
	10	1190	soybeans	0.96	20.0	4.6	100.0
	13	1360	(2 weeks)	1.04	22.4	3.3	83.3
	14	1650		0.80*	22.1	3.9	75.8
	15	1800		0.80	23.4	3.9	70.1
May 16	2	1540	Cooked	0.77	21.0	3.3	100.0
	3	1760	soybeans	0.69	20.0	4.7	95.2
	4	1580	(3 weeks)	0.60	21.3	4.1	80.0
	6	1655		0.60*	15.4	4.0	77.0
	7	2020		0.62	18.9	4.0	83.3
	8	1870		0.65	20.3	3.4	111.1
	9	1660	Raw	0.73	17.5	4.2	62.5
	10	1490	soybeans	0.60	17.2	4.6	86.9
	11	1800	(3 weeks)	0.77	21.7	6.5	100.0
	13	1470		0.69*	20.0	3.3	80.0
	14	1860		0.60	19.3	3.8	77.0
	15	1830		0.60	15.4	3.8	66.6

*Determinations made according to Folin's new method (1930). Even with this sensitive test it was necessary to use 10 cc. instead of 5 cc. filtrate to obtain sufficient color for comparison.

TABLE II.

Average values in mg. per 100 cc. blood serum.

Food	Uric acid	Urea N	Inorganic P	Cholesterol	Reference
Millet and cabbage	2.79	18.3	5.31	57.5	Horvath
Raw soybeans	3.23	37.6	5.89	72.7	
Millet and cabbage	Trace	23.9	3.80	70.9	Tso and Ling
Raw soybeans	"	20.2	4.27	80.7	(Present paper)
Cooked soybeans	"	20.8	3.91	86.6	

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The Action of Ephedrine on Melanophores.

HSIANG-CH'UAN HOU. (Introduced by B. E. Read.)

From the Summer Institute for Biological Research, Amoy, and the Department of Pharmacology, Peiping Union Medical College.

Nadler[1] found that in the squid, *Loligo pealii,* ephedrine injected subcutaneously, produced a local blanching which was considered as due to an inhibition of the smooth muscle of the chromatophore system and a generalized reddish coloration ascribed to a stimulation of the central nervous system. These experiments have been repeated on several other kinds of fish, namely *Cyprinus rubrofuscus, Mylopharyngodon aethiops, Cyprinus abbreviatus* and *Ophiocephalus argus* and they all responded by a generalized paleness. Examination of the scales showed that all the melanophores were contracted. One fish remained in such state for 36 hours. It appears therefore that, in the fresh water fish studied, ephedrine does not exert a double action, or both the peripheral and the central actions bring about a similar change, namely contraction of the black pigment cells. Experiments with adrenalin appear to substantiate the latter view, since this drug caused only a local blanching and no generalized paleness of the scales.

The generalized contraction of the melanophores caused by ephedrine has been found to be true also in the frog, *Rana nigromaculata.* Injection into the dorsal lymph sac of 20 mg. per kilo caused the skin of the frog to remain pale for 6 hours.

Other observations on small, salt water fish, *Trigentiger bifasciatus* and *Microcanthus serrus,* with a few drops of ephedrine solution added to the surrounding sea water showed in a minute or even less a generalized blanching and the melanophores began to contract. The contraction gradually became more marked and in 15 to 20 minutes most of the black pigment cells contracted to their minimal sizes. This contraction of the melanophores usually lasted from one half hour to 2 hours, after the fish had been transferred to ordinary sea water, depending upon the time of the previous immersion in the ephedrine-sea water. Adrenalin in sublethal dose produced little or no such effect.

That there is a local action of the ephedrine is further supported from the experiment by Barbour and Spaeth[2] with isolated scales of *Fundulus heteroclitus.*

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[1] Nadler, J. E., *Chinese J. Physiol.,* 1927, **1**, 271.

[2] Barbour, H. G., and Spaeth, R. A., *J. Pharmacol. and Exp. Therap.,* 1917, **9**, 356.

The findings obtained with isolated fish scales of fresh water fish with the drugs other than ephedrine and pseudoephedrine were essentially the same as those obtained by Barbour and Spaeth,[2] except pilocarpine, which showed a contraction instead of expansion and, in the case of *Ophiocephalus argus,* expansion with $BaCl^2$ and contraction with pituitrin instead of contraction and expansion respectively. This difference might be peculiar only to certain fish.

The degree of contraction or expansion of the individual cells and the number of cells undergoing either of these changes varies with the time of immersion and the drug used, some having a much quicker action than others. Table I illustrates the point.

TABLE I.
Isolated scale of Mylopharyngodon aethiops.

	2 min.	5 min.	10 min.	30 min.	1 hour	2 hours
Ephedrine 0.1%	2 C	3 C	4 C	4 C	4 C	3 C
" 1.0%	1 C	2 C	2 C	2 C	4 C	4 C
Pseudoephedrine 0.1%	2 C	2 C	1 C	1 E	2 E	3 E
" 1.0%	1 C	1 C	1 E	4 E	4 E	4 E
$BaCl_2$ 0.1%	1 C	1 C	2 C	2 C	3 C	3 C
" 1.0%	1 C	2 C	3 C	3 C	4 C	4 C
Pituitrin	B	1 E	2 E	2 E	3 E	3 E
Adrenaline 1:10,000	2 C	4 C	4 C	4 C	4 C	4 C
NaCl	2 E	2 E	4 E	4 E	4 E	4 E
KCl	2 C	4 C	4 C	4 C	4 C	4 C
Water (distilled)	1 C	1 C	2 C	3 C	4 C	4 C

E denotes expansion, C contraction, B balanced, and the figure in front of the letter the extent of contraction or expansion, 4 being maximal.

I have also repeated Spaeth and Barbour's experiment[3] with ergotoxine and found that it reversed the action of ephedrine. Spaeth and Barbour obtained a similar effect with adrenalin. They assume that ergotoxine acts on the myoneural junction. If so, then the site of action of ephedrine on the isolated melanophores is at the myoneural junction also.

With atropinization it was found that the melanophores still contract toward ephedrine even when the scales were previously treated with atropine. This action again is similar to that of adrenalin.

[3] Spaeth, R. A., and Barbour, H. G., *J. Pharmacol. and Exp. Therap.,* 1917, **9**, 431.

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Anaphylaxis With Water Soluble Specific Substance from Yeast-Like Fungi.

T. J. KUROTCHKIN AND C. E. LIM. (Introduced by Hsien Wu.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

Most fungi including yeast-like forms such as Monilia, are known to possess slight antigenic power. On the other hand, the clinical symptoms manifested in certain fungous infections suggest that hypersensitivity may play a rôle in such infections. It would, therefore, seem reasonable to assume that the antigenic and sensitizing properties of the fungi may represent 2 different biological faculties not necessarily associated with each other. The present experiment has been conducted to find some evidence in support of this view.

The fungi used were *Monilia psilosis, Monilia pinoyi* and Saccharomyces. Water soluble specific substances of sufficient activity in precipitation and passive anaphylaxis tests and free from antigenic power were prepared from each strain of organisms using the alkaline method.[1] For active sensitization of guinea pigs heat-killed cultures of the fungi were used. All 3 organisms were grown upon Sabouraud glucose agar for 48 hours, both monilia being kept at 37°C. and Saccharomyces at room temperature. The growth was then washed off with normal saline, 10 cc. of which was used for each agar slant, and heated to 75°C. for 30 minutes before use for injections. Each dose was equal to 1/10 of an agar slant suspended in 1 cc. of saline and injected intraperitoneally.

In order to determine the number of injections necessary for effective sensitization of guinea pigs, a series of these animals, 4-5 in number, received one, 2 or 3 injections respectively of the heat killed fungus cultures. At the end of 2 weeks all animals were tested for hypersensitivity by giving them intravenous injections of the homologous specific substance from 0.5 to 4 mg. dissolved in one cc. of normal saline.

It was found that both monilia cultures produced active sensitization of guinea pigs as evidenced by the typical anaphylactic shock resulting from injection of the specific substance. In those instances in which the shock ended fatally a marked distension of the lung occurred. As shown in Table I the sensitizing capacity of both monilia was somewhat different. Successful sensitization with the

[1] Tomcsik, J., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 810.

TABLE I.

Active Sensitization of Guinea Pigs with Yeast-like Fungi.

Series	Sensitized with	Doses of fungus used	Homologous water soluble specific substance mgm.	Symptoms	Result
1	*Monilia pinoyi*	1	4 2 1	No symptoms Severe shock No symptoms	Survived " "
2	"	2	2 1 0.5	Severe shock " " No symptoms	Dead in 2-4 min. " " 3-4 " Survived
3	"	3	2 1 0.5	Severe shock " " No symptoms	Dead in 2-4 min. " " 2-3 " Survived
4	*Monilia psilosis*	1	4 2 1.5	" " " " " "	" " "
5	"	2	4 2 1	" " " " " "	" " "
6	"	3	2 2 2	Slight shock Severe shock Moderate shock	" " "

cultures of *Monilia psilosis* was attained only after the administration of at least 3 intraperitoneal injections, whereas a single injection of the *Monilia pinoyi* culture was sufficient to produce sensitization. In this latter case, the shock developed only when 1 or 2 mgm. of the substance were given. It will be noted that the development of the shock was largely dependent upon the amount of the substance given, and this suggests the existence of a certain optimal relationship between the degree of sensitization and anaphylactizing substance.

The attempt to sensitize guinea pigs with heat-killed cultures of the Saccharomyces gave completely negative results. Several series of the animals were injected with the cultures of this fungus, the doses ranging from one to 5. Subsequent injection of these animals with the water soluble substance derived from Saccharomyces produced no effect whatsoever. This indicates clearly that the particular strain of Saccharomyces used in our work has no sensitizing power in active anaphylaxis.

The striking difference in sensitizing capacity between Monilia and Saccharomyces on the one hand, and that between the monilias themselves suggests that this property may in some way be con-

nected with the morphological structures of the organisms studied here. Monilia is known to be differentiated from true yeast by the inability of the former fungus to produce asci and by its tendency to grow in a filamentous form, especially when cultivated in liquid media. With the purpose of elucidating this point the following experiment was performed. *Monilia psilosis,* the sensitizing power of which is moderate, as indicated in the previous experiment, was grown in glucose broth for 5 days. During this time the fungus produced abundant mycelial growth. This was centrifuged, washed, suspended in saline and heated. A control suspension of the same organism was prepared using 48-hour agar culture which contained mainly budding cells. The density of both suspensions was then adjusted to an approximately even degree and a series of guinea pigs were sensitized with both suspensions of the monilias respectively. These animals were tested 14 days later for hypersensitivity in a similar manner. It was found that while the suspension prepared from agar growth failed to produce sensitization after 2 injections, a single injection of broth culture was sufficient for this purpose, as shown in Table II.

TABLE II.

Sensitizing Power of Mycelial and Budding Cell Growths of Monilia Psilosis.

Series	Sensitized with *Monilia psilosis*	Water soluble specific substance mgm.	Symptoms	Result
1	Agar culture 1 dose	2	No symptoms	Survived
		1	" "	"
2	" 2 doses	2	" "	"
		1	" "	"
3	Broth culture 1 dose	2	Slight shock	"
		1	No symptoms	"
4	" 2 doses	2	Severe shock	Dead in 5-6 min.
		1	Moderate shock	Survived

From the experiment it may be concluded that the sensitizing power of the mycelial growth is distinctly higher than that of the budding cell growth of the same fungus. It also explains our finding that *Monilia pinoyi* exhibited higher sensitizing capacity than *Monilia psilosis;* culturally, the former fungus differed from the latter in that its growth even upon solid medium was accompanied by rapid and rich formation of the mycelium.

5242

Titration of Diphtheria Toxin and Antitoxin on Hamsters.

FAN CHUAN AND C. E. LIM. (Introduced by Hsien Wu.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College.

The use of guinea pigs in the titration of diphtheria toxin and antitoxin, while being a standard method, is not without its drawbacks. The animal is not always procurable and its cost of purchase and maintenance is comparatively high. The Ramon precipitation method is simple and economical, but the results are not always quite satisfactory or conclusive. Furthermore, we are not without misgivings in the titration of a biological phenomenon by purely chemico-physical means, at least not without further understanding of the nature of their correlation.

In an attempt to discover a method of titration which can be applied when guinea pigs are not available, we find that the results obtained by standardizing diphtheria toxin and antitoxin on hamsters are comparable to those obtained by the use of guinea pigs.

Throughout the experiment hamsters, *Cricetulus griseus*, weighing from 25 to 30 gm. are used. They are easily obtainable in this part of the world and are quite susceptible to diphtheria toxin. The diphtheria toxin we use is from the National Epidemic Prevention Bureau, Peiping. Its M.L.D. for guinea pig is 0.005 cc. and its L + dose 0.22 cc. The antitoxin is obtained from the same source and checked by us against the standard unit provided by the U. S. Hygienic Laboratory, Washington, as containing 350 units per cc. They are diluted to the required concentrations with 0.9% saline. In case both toxin and antitoxin are injected into the same animal, they are always mixed *in vitro* and allowed to stand one hour before injection. The total amount injected is 1 cc. and all injections are given subcutaneously.

Three sets of experiments are performed. One sample test from each set is reported in this communication.

The first set of experiments is to determine the minimal lethal dose for hamsters in terms of the M.L.D for guinea pigs. The smallest dose that will kill a hamster at the end of 3 days is found to be equivalent to 1/6 of a guinea pig M.L.D. as shown in the tabulation below:

Dosage of Toxin		Days of Survival
1.6	M.L.D.	1
0.5	"	2
0.25	"	2
0.20	"	2¼
0.18	"	2½
0.166 (1/6)	"	3
0.15	"	Survived
0.14	"	"

The second set is to titrate a sample of antitoxin against toxin of known potency. Various amounts of antitoxin are mixed with 1/6 of a L + dose before injection. It is found that the amount of antitoxin that will delay the death of the hamster till the end of the third day after injection is 7/42 or 1/6 unit as given in Table I. It happens, in this case, as we have already shown that the minimal lethal dose for a hamster is 1/6 of a guinea pig M.L.D. Therefore, if we take it as a basis, we can calculate the potency of the antitoxin easily. Here 0.5 cc. of the antitoxin diluted 1:1050 protects the hamster from death till the end of the third day. If that amount contains 1/6 L + dose, the undiluted antitoxin must contain 350 units per cc.

TABLE I.
Titration of Diphtheria Antitoxin on Hamsters.

Antitoxin		Toxin		Days of Survival
Dilution	Equivalent in 0.5 cc.	Dilution	Equivalent in 0.5 cc.	
1: 750	7/30 units	1.1:15	1/6 L + dose	Survived
1: 900	7/36 "	"	"	11
1:1050	7/42 "	"	"	3
1:1200	7/48 "	"	"	2½
1:1350	7/54 "	"	"	2

Similarly, toxin can be titrated by mixing various amounts of its L + dose with an equivalent amount of antitoxin unit. Thus in Table II the amount of toxin that kills the hamster at the end of 3 days in the presence of, say, 1/6 unit of antitoxin is 1/6 L + dose. If we take this as the point of neutralization, we can calculate the L + dose for guinea pig. Since 0.5 cc. of the toxin diluted 1.1:15 contains 1/6 L + dose, the L + dose must be 0.22 cc. of the original toxin. This composite table, based upon the result of a large number of tests, also shows that the hamsters respond quite consistently to the toxin antitoxin mixture of different proportions. From this tabulation, it is also evident that with a known unit of antitoxin the L + dose of the toxin can be titrated, and similarly, with a known L + dose the antitoxin unit can be determined.

TABLE II.

Titration of Diphtheria Toxin and Antitoxin on Hamsters.

Antitoxin in Standard Unit	Toxin in Guinea Pig L + Doses				
	1/8	1/7	1/6	1/5	1/4
1/8	D S	D D	D D	D D	D D
1/7	S S	D D	D D	D D	D D
1/6	S S	S S	D D	D D	D D
1/5	S S	S S	S S	D D	D D
1/4	S S	S S	S S	D D	D D

S—Living at the end of 3 days. D—Dead at the end of third day.

These experiments indicate that hamsters may be used for the titration of diphtheria toxin and antitoxin for preliminary tests when guinea pigs are not available.

5243

The Determination of Glycogen in Tissue.

ALBERT CARRUTHERS. (Introduced by Hsien Wu.)

From the Department of Biochemistry, Peiping Union Medical College.

The method in general use for the determination of glycogen in tissue[1] involves the use of strong KOH to effect a separation of glycogen from proteins. Other methods[2] for treatment of tissue have not proved satisfactory.

A suitable method, however, has been found by treating boiling water extracts of tissue (liver only has been employed thus far) with trichloracetic acid. A filtrate from this mixture gives quantitative values for tissue glycogen. The use of KOH is rendered unnecessary and the procedure saves considerable time, particularly when amounts of tissue of more than a gram are involved.

Procedure: Tissue is weighed and plunged into boiling water for about 2 minutes when it is ground thoroughly and re-boiled. The

[1] Pflüger, E., *Arch. ges. Physiol.*, 1909, **129**, 362.

[2] Fränkel, S., *Arch. ges. Physiol.*, 1892, **52**, 125.

mixture is cooled and diluted to a known volume and after mixing it is centrifuged. The creamy supernatant fluid is removed (the bulk of the proteins are insoluble) and an aliquot part added to a definite volume of trichloracetic acid (we have employed an equal volume), allowed to stand 15 minutes and filtered. Trichloracetic acid of strengths varying from 2 to 5% has been found satisfactory but with 2% acid it has been necessary to refilter. A second filtration has in this case always given satisfactory solutions. To an aliquot part of the filtrate 3 volumes of alcohol and a pinch of N.Cl are added, and the mixture is allowed to stand, preferably overnight. The deposit obtained after centrifuging is a clean white precipitate and the alcoholic fluid is perfectly clear. The deposit is washed twice with 80% alcohol (containing NaCl), once with 95% alcohol, once with absolute alcohol and once with ether. The precipitate is dissolved in water and hydrolyzed. Three methods, Folin-Wu,[3] Folin-Wu,[4] and Hagedorn and Jensen[5] have given identical values for the glucose in the hydrolysates. The values obtained by this method have been compared with those given (a) after treating the 75% alcohol precipitate with 60% KOH and reprecipitating with alcohol, (b) after adding alcohol to the aqueous extract to make 75%, and proceeding as in (a), and (c) by treating a sample of the same tissue with alcohol, and then proceeding as in (a). Identical values for glycogen have been obtained.

The limits of the method have not yet been worked out, but satisfactory results are obtained when about 5 gm. of liver tissue are extracted with water and the volume made to 50 cc. The glycogen in this volume has varied from 150-200 mg.; that is, the original tissue has contained 3-4% of glycogen. It is desirable to keep the volume of the aqueous extract reasonably low to facilitate complete precipitation of glycogen subsequently.

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[3] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[4] Folin, O., and Wu, H., *J. Biol. Chem.*, 1920, **41**, 367.

[5] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

5244

A New Type of Gas Burette.

HSIEN WU.

From the Department of Biochemistry, Peiping Union Medical College.

Gas burettes reading directly to 0.01 cc., and by estimation to 0.001 cc., are used in certain kinds of apparatus, for example, that of Haldane. A serious limitation of such burettes is that the fine graduation covers only a small fraction of the total volume. In a 10 cc. Haldane's apparatus, the fine graduation usually extends only from 7 to 10 cc. Analysis of a mixture of gas containing more than 30% of a gas to be determined by absorption cannot be made with the apparatus in the usual manner. Nor can samples less than 7 cc. be taken for analysis.

If the fine graduation is extended to the whole of the burette, even with a capacity of only 10 cc., it would have to be more than a meter long. This is, of course, impractical.

In designing a special apparatus for the study of gas equilibria in blood, we have developed a new type of burette which permits reading of volume from 0.1 to 50 cc. or more directly to 0.01 cc. The length of the burette need not exceed 25 cm. Such a burette can, of course, be incorporated into any apparatus.

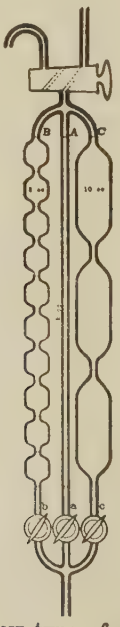

FIG. 1. A new type of gas burette.

The principal feature is the division of the total volume into 2 or more portions. The burette shown in the accompanying figure has a capacity of 50 cc. and is divided into 3 arms. Arm A has a capacity of 2 cc., or preferably a little more, and is graduated to 0.01 cc. Arm B consists of ten 2-cc. bulbs, while Arm C consists of three 10-cc. bulbs. These bulbs are conveniently blown out of a 2 mm. capillary tubing. The 3 arms are joined together above the graduations by a 2-mm. capillary tubing. The volume of this connecting piece need not exceed 0.1 cc. The constrictions in Arms B and C have the same internal diameter as Arm A. The lower ends of the arms, provided with cocks, are joined together and connected to a manometer and a levelling bulb.

For a 10 cc. burette 2 arms will suffice. One arm consists of nine 1-cc. bulbs while the other arm is a 1 cc. burette graduated to 0.01 cc.

With Arms A and B filled with mercury to the zero mark, cocks a and b closed, and c open, the gas to be measured is introduced into the burette. If the volume of the gas, estimated in Arm C is, for example, between 20 and 30 cc., the mercury in C is brought to the 20 cc. mark, cock c is closed and cock b is opened. If the reading in B is between 8 and 10 cc., the mercury is brought to the 8 cc. mark, b is closed and a is opened. The final reading of the volume is taken on arm A. The total volume of the gas measured is, of course, the sum of the volumes in the 3 arms plus that of the connecting piece above the graduations.

5245

Transmission of Kala-Azar to Hamsters (Cricetulus griseus) by the Oral Route.

O. K. KHAW. (Introduced by James R. Cash.)

From the Division of Parasitology, Department of Pathology, Peiping Union Medical College.

Prior to 1928, probably only 4 flawless successful experimental transmissions of kala-azar *per os* were recorded in literature. Monkeys were employed by Archibald,[1] who used infected human material, and also by Greig and Christophers,[2] who injected by means of a hypodermic syringe human material obtained by splenic puncture and cultures of *Leishmania donovani* into the jejunum. Tran-

[1] Archibald, A. C., *J. R. A. M. C.*, 1914, **23**, 479.

[2] Greig, E. D. W., and Christophers, S. R., *Ind. J. Med. Res.*, 1916, **13**, 151.

sient infection was obtained by Cornwall and La Frenais,[3] who fed bread soaked in cultures of *Leishmania donovani* to a white rat. Cultures also produced infection when fed to a white mouse, the subject of experiment by Christophers, Shortt and Barraud.[4]

Using Chinese hamsters *(Cricetulus griseus)*, Shortt, Craighead and their colleagues reported[5, 6] 9 successes by feeding emulsion of infected organs. Their culture experiments were also positive. Young, Smyly and Brown,[7] however, failed twice to produce infection in the same rodents by feeding infected spleen and liver. Young, Hertig and Liu[8] did not fare better when they fed whole carcasses of hamsters infected with kala-azar to normal ones during a period of 47 days. It is with the object of checking the conflicting results of these workers that the present investigation was undertaken.

After 12 hours' fasting, 30 normal hamsters were each fed by means of a pipette 0.5 cc. of a thick saline emulsion prepared from the spleens and livers of 4 hamsters heavily infected with kala-azar. This finely ground emulsion, swarming with *Leishmania donovani* was readily swallowed by the hungry animals.

Four (F-1 to F-4) of them were killed at half hourly intervals after feeding, another lot of 4 (F-5 to F-8) at 1, 2, 2, 3 hour periods, 7 (F-9 to F-15) after one day, 6 (F-16 to F-22) at weekly intervals and the remaining 9 animals allowed to die naturally.

Examination of gastric and intestinal contents was discontinued after the 7th day but heart's blood, spleen, liver, abdominal and cervical glands, bone-marrow, testes, kidney, and brain were examined during the entire experiment for Leishman-donovan bodies both by staining and culture. The gastric and intestinal contents of 12 (F-1 to F-12) hamsters killed during the first 4 days after feeding were, in addition, examined immediately after death. The results of these studies are summarized in Table 1.

Leishman-donovan bodies in flagellate forms were for the first time detected on the 37th day in a culture of spleen of a hamster killed 7 days after the feeding. Previous to this, culture and smears from all hamsters killed during the first 7 days after feeding (F-1 to

[3] Cornwall, J. W., and La Frenais, H. M., *Ind. J. Med. Res.*, 1916, **3**, 698.

[4] Christophers, S. R., Shortt, H. E., and Barraud, P. J., *Ind. Med. Res. Memoir*, 1926, **4**, 89.

[5] Shortt, H. E., Craighead, A. C., Smith, R. O. A., and Swaminath, C. S., *Ind. J. Med. Res.*, 1928, **16**, 271.

[6] Shortt, H. E., Craighead, A. C., and Swaminath, C. S., *Ind. J. Med. Res.*, 1929, **17**, 335.

[7] Young, C. W., Smyly, H. J., and Brown, C., *Am. J. Hyg.*, 1926, **6**, 254.

[8] Young, C. W., Hertig, M., and Liu, P. Y., *Am. J. Hyg.*, 1929, **10**, 183.

TABLE I.

Hamster No.	Days after feeding	Splenic enlargement	Organs positive for L. D. bodies		Remarks
			Stained smears	Cultures	
F-1 to F-14	½ hr. to 6 days	0	0	0	Cultures kept incubating for 46 to 51 days
F-15	7	0	0	*Spleen* on 37th day	Other organs neg. Cultures kept for 48 days
F-16	12	+	0	Spleen on 14th day	Ditto
F-17	19	0	0	Spleen (25th day) *Blood* (36th day)	,,
F-18	26	+	Spleen	0	Cultures kept for 53 days
F-19	34	+	*Liver, bone-marrow*	Spleen (10th day)	Liver culture contaminated
F-20	45	+	Spleen	Spleen (15th day)	Cultures kept for 34 days
F-22	51	+	Spleen, liver, m.g. bone-marrow, kidney	Spleen, blood, mesenteric glands	
F-23	11 mos.	0 (putrid)	Mesenteric glands, *kidney*	Cultures contaminated (F-23)	
F-24	142	?	Spleen, liver	*Joint* (12th day)	Other cultures contaminated
F-25	143	+	Spleen, liver, bone-marrow, blood, m. glands, brain, *spinal cord*	*Brain* (21st day) *testis* (9th day)	Ditto
F-26	131	+	Spleen, liver, bone-marrow, blood, kidney, brain, joint	Cultures contaminated	,,
F-27	177	+	Ditto (in addition, testes)	Spleen	,,
F-28	95	+	Spl., liver, b.-m., blood, brain	Spleen, liver, kidney	
F-21	7	0	0	0	Cultures kept for 72 days
F-29	Escaped				
F-30	319	Slight	0	?	

F-14 and F-21) showed no parasites, though the cultures from their organs were incubated from 46 to 51 days, during which time 3 examinations of each culture were made. Twelve hamsters killed or dying from 7 to 319 days after being fed with parasites showed *Leishmania donovani* either in smears or cultures or both. Animals dying naturally showed enormous splenic enlargement. One hamster (F-23) was too decomposed for examination. Another (F-29) escaped. The last (F-30), sacrificed (Oct. 29, 1930) after 319 days, had a moderately enlarged spleen but the smears were negative for parasites; the result of the cultures is yet unknown.

From examinations of stomach and intestinal contents (fresh and stained), it was noted that though liver tissue was distinguishable up to 24 hours, Leishman-donovan bodies, even one hour after ingestion, could be made out with difficulty. A few degenerated ghosts of parasites were seen in an eighth hour hamster. No flagellate forms were observed.

The cervical glands of 7 hamsters (F-8 to F-14) under observation from the tenth hour to the sixth day were found to be of normal size and free from parasites, indicating that no injury to the oral mucosa was inflicted during the feeding process. The mesenteric glands were negative up to the fifty-first day although the infection of bone-marrow and kidney at this time shows generalized parasitization.

Conclusion. Excluding the 14 hamsters sacrificed within the first week, F-23 which was too much decomposed for examination and F-29 which escaped, the *per os* experiment registers an infection of 12 out of 14 hamsters—a result comparable to that (9 out of 11) obtained by the Indian workers. Young and his associates in Peiping, working with the same parasites used in these experiments, may have failed to produce infection *per os* because they did not grind and dilute the organs used, thereby facilitating phagocytosis and absorption; furthermore, they made no cultures of the organs of animals fed with *Leishmania donovani*.

In these experiments, evidence of infection became apparent on the 7th day after feeding parasites and generalized parasitization was found on the 51st day. The majority (5 out of 7) died within the period of 100 to 200 days, the course of the infection being about the same as that after infection by the intraperitoneal route with very dilute emulsion of kala-azar organs.

No flagellate forms were noted in the contents of the gastro-intestinal tract in an observation on 12 hamsters extended from half an hour to 4 days. This is to be expected as the medium was contaminated and temperature too high for transformation into flagellates.

5246

Spontaneous and Experimental Infection of Pigeons with B. Aertrycke.

J. R. CASH AND C. A. DOAN.

From the Laboratories of the Rockefeller Institute for Medical Research, New York City, and the Department of Pathology of the Peiping Union Medical College, Peiping, China.

In the course of some studies upon blood-formation in pigeons, which had first been rendered anemic by fasting and then maintained upon various diets, several birds showed total white blood-cell counts varying from 60,000 to 187,000 cells per cmm. In the blood of these pigeons, myelocytes, varying from 2% to 70%, appeared. Coincident with the development of this striking change of the myeloid elements of the blood, these pigeons became acutely ill, rapidly developed an extreme degree of anemia (1,200,000 to 1,900,000 erythrocytes per cmm.) and died within less than a week after the apparent onset of their illness. In a series of 52 pigeons, 7 birds died in this manner.

Autopsies showed marked enlargement of the liver, spleen, and kidneys and massive hyperplasia of the bone-marrow. The liver and kidneys were studded with extensive infiltrations of myelocytes in which extremely young forms, frequently in mitosis, were present. In addition, large numbers of mononuclear phagocytes, frequently forming nodules, were found in the liver, spleen, kidneys, and bone-marrow. In these latter lesions numerous small, Gram-negative bacilli were regularly present, but no bacteria were ever demonstrated in the accumulations of myelocytes seen in the liver and kidneys. In none of the other 45 pigeons of the series were changes like these noted.

No adequate bacteriological studies were done upon the first 3 birds showing these lesions, but cultures of the blood, liver, kidney, and bone-marrow of the fourth bird (Pigeon 86) all gave pure growths of a small, Gram-negative bacillus, identified by Dr. L. T. Webster as *B. aertrycke*. An identical organism was recovered from the other 3 birds spontaneously developing the same disease.

The blood and tissues of Pigeon 86 were inoculated into 8 well-nourished, apparently normal pigeons. Three birds received 1 cc. each of blood given intravenously, two 2 cc. each of a heavy saline emulsion of liver, two 2 cc. each of a similar emulsion of bone-marrow, and a single bird was injected with 2 cc. of emulsion prepared from the kidney. One of the pigeons which had been inoculated

with the liver-emulsion became ill within 24 hours and died on the fifth day after inoculation. Though the total leucocyte count of this bird never exceeded 35,000 cells per cmm., the myelocytes varied from 36% to 71% during the course of its illness. At autopsy, lesions identical with those of Pigeon 86 were found and *B. aertrycke* in pure culture was recovered from the liver. None of the other 7 pigeons showed any evidence of disease, although they were observed for a month before being killed for study. It is of interest, however, that *B. aertrycke* was grown from the livers of 3 of them, although no lesions were present. Cultures made from the livers of the other 3 remained sterile.

Broth cultures of the organism *(B. aertrycke)* originally isolated from the liver of Pigeon 86 have proven highly pathogenic for pigeons when injected intraperitoneally, 0.5 cc. of a 24-hour culture causing death within 48 hours. Five such birds all developed moderately high leucocytosis but showed no myelocytes in the peripheral blood. At autopsy, intense fibrinopurulent peritonitis, acute splenic tumor, cloudy swelling of the liver and kidneys, and early, but well-marked, myeloid hyperplasia of the bone-marrow were found; *B. aertrycke* was readily recovered from all 5 birds.

Seven normal pigeons, whose blood previously had been studied and also found to contain no agglutinins for *B. aertrycke,* were fed with 24-hour broth culture of the original strain of *B. aertrycke,* 1 cc. being administered to each bird, drop by drop, from a small pipette. Within 48 hours, all developed loss of appetite, diarrhea, and leucocytosis. After an illness lasting from 9 to 27 days, 4 died and 3 recovered. During this time the individual birds developed leucocytoses varying from 60,000 to 162,000 per cmm., due mainly to an increase in number of myeloid cells but also in part produced by a well-marked rise in monocytes. Myelocytes appeared in numbers varying from 1% to 10%. There was a moderate reduction in number of lymphocytes. Those birds which died had developed a much greater degree of anemia than those which recovered, but in other respects no well-marked difference was apparent in the blood-pictures of the 2 groups. At autopsy, the 4 pigeons which died showed enormous myeloid hyperplasia of the bone-marrow, extensive infiltrations of myelocytes throughout the liver and kidneys, as well as all of the other lesions which have been described above in birds spontaneously developing what appeared to be the same disease. In addition, 3 of these pigeons showed lobular pneumonia which was clearly due to bacteria aspirated at the time of feeding. No lesions were present in the intestines of any of them. *B. aertrycke*

was isolated in pure culture from the liver of all 4 birds, from the pneumonic lesions of each of the 3 cases in which they occurred, but from the intestine of only one bird. Cultures of the intestine of the other 3 gave colon bacilli. All cultures were made immediately after death.

One month after oral administration of *B. aertrycke,* the sera of the 3 birds which recovered were tested for agglutinins to this organism. None was found in one case, but the other 2 agglutinated the bacteria in dilutions of 1/1280 and 1/2560 respectively. At the end of 2 months the blood pictures and weights of all 3 pigeons had returned to normal. They have been observed now for 5 months and have shown no manifestations of disease.

Although it seemed highly probable that the organism with which we were dealing was responsible for these striking disturbances of the myeloid cells, it was obviously necessary to rule out the presence of some filterable forms of microorganism in our cultures before drawing this conclusion. Accordingly, each of 6 normal pigeons was fed 2 cc. of a bacteria-free filtrate prepared by passing a 24-hour broth culture of *B. aertrycke* through a VV Berkefeld filter. To test the sterility of this filtrate, 40 cc. of it, taken from that with which the birds were fed, was inoculated into 100 cc. of sterile broth. No growth took place within 48 hours. None of these 6 pigeons showed any loss of weight, changes in the blood or other manifestations of disease for one month after oral administration of filtrate. They were then killed for study but no gross or microscopical lesions other than certain minor changes frequently observed in apparently normal pigeons were found. No agglutinins for *B. aertrycke* were found in the blood of any of them; cultures of the liver were sterile in all instances and no pathogenic organisms were isolated from the intestine. The pathogenicity of the broth culture of *B. aertrycke* from which the filtrate was prepared was tested by feeding it in amounts of 1 cc. to each of 5 normal pigeons. All 5 birds developed characteristic symptoms of infection with this organism. Two of them died, showing all of the lesions which have been described and *B. aertrycke* was readily recovered in both instances. The other 3 survived but showed high agglutination titers to *B. aertrycke* a month after having been fed with this organism.

We therefore offer these experiments as evidence of the following points: 1. The spontaneous development in under-nourished pigeons of a fatal disease associated with marked myeloid hyperplasia of the bone marrow, striking increase of the myeloid elements of the blood, and extensive infiltration of the liver and kidneys with mye-

loid tissue. 2. The cultivation of a bacillus from such pigeons, which has been identified as *B. aertrycke*. 3. The experimental production in pigeons of apparently the same disease and identical lesions by injection of liver-emulsion of an infected bird or the oral administration of cultures of this organism. 4. Bacteria-free filtrate of *B. aertrycke* produces no demonstrable effect when fed in large, single doses to normal pigeons.

Though the relationship of this disease to fowl leucemia is one of considerable interest, we can offer no further comment on this question since we have not yet had the opportunity of studying the latter disease. Though the literature contains many reports of unusually high leucocytoses in birds, we have been unable to find any instance in which large numbers of myelocytes in the peripheral blood or heterotopic formation of myeloid cells, as we have observed, have resulted from bacterial infection. Further studies now in progress upon this subject will be reported at a later date.

Iowa Section.

State University of Iowa, November 6, 1930.

5247

Effect of Diverting the Portal Circulation Upon Toxicity of Proteose Fractions.

J. T. McCLINTOCK and H. M. HINES.

From the Department of Physiology, State University of Iowa.

Certain similarities in the pathological changes in the intestine caused by acute ligation of the portal vein to those produced by a lethal dose of toxic proteose fractions suggested to us the following experiments. Toxic material was extracted from the mucosa and content of closed intestinal loops. Its toxicity was tested by giving an intravenous injection of aliquot portions of the material to 4 normal control dogs. All 4 controls developed the characteristic symptoms of vomiting, bloody diarrhea, prostration and coma. Death took place within 10 hours after the injection. At post mortem the mucosa of the small intestine was found to have the typical hemorrhagic condition. Aliquot portions of the same material were injected into 4 dogs in which the portal circulation had been diverted from the liver through collateral veins directly into the systemic circulation by a 2-stage ligation of the main trunk of portal vein.[1] Three of the dogs with portal ligation survived the lethal dose without showing the symptoms of toxemia usually seen after such injections. One of the survivors was sacrificed 48 hours later and the mucosa of the small intestine appeared entirely normal. Three months later one of those which had survived was given a second lethal dose of material of proven toxicity and it again failed to show the usual symptoms. The fourth dog with a diverted portal circulation showed the usual symptoms and died within 15 hours after receiving the toxic material. The characteristic hemorrhagic mucosa was found at post mortem but because of adhesions it was impos-

[1] This modification of the Eck fistula was demonstrated by L. R. Dragstedt at 1930 meeting of Federated Biological Societies.

sible to make certain of a complete diversion of the portal circulation.

The fact that 3 dogs out of 4 with their portal circulation diverted from the liver survived injections of a lethal dose of a toxic proteose fraction is strongly indicative of a more or less complete acute hepatic portal occlusion as the probable cause of the bloody diarrhea and hemorrhagic mucosa which results from such injections. It is believed that this intestinal condition is a major factor in causing the death of such animals.

5248

Carotene from Lettuce and Its Relation to Vitamin A.

H. S. OLCOVICH AND H. A. MATTILL.

From the Biochemical Laboratory, State University of Iowa.

Carotene was obtained from a methyl alcohol solution of the unsaponifiable fraction of the lipids of lettuce. After recrystallization from petroleum ether, the melting point was 179.5-180°. 200 mg. were obtained from approximately 150 kilos of fresh lettuce.

The crystal form differs depending on the solvent from which it is crystallized: from petroleum ether, rhombohedrons; chloroform and methyl alcohol, needles; carbon disulfide and absolute alcohol, clusters of needles; petroleum ether and methyl alcohol, square plates; acetone, triangular plates. The different crystal habits are all members of the hexagonal system.

On long standing at room temperature, or on heating for 24 hours at 105°, the crystals bleach, without losing their form. This fading is from the outer edge inward, showing a sharp line of demarcation between the unchanged carotene and the bleached or "achroo-carotene". There is no change in weight, and the bleaching is accomplished as easily in nitrogen as in oxygen. Formerly hexagonal, the crystals are now isometric, denoting a radical change in the molecular structure. Achroo-carotene has no physiological activity.

Carotene is efficacious in curing the xerophthalmia, and allowing a resumption of growth, in vitamin A deficient rats. 0.005 mg. per day seems to be the practical minimum dose, although several individual rats have been able to grow on less. The effect of giving carotene continues for some time after the dosage has been stopped, and there seems to be a quantitative relationship between the amount

given and the weight increase obtained, whether the carotene be given in one dose or in divided doses. 0.005 mg. allows a growth of at least 2 gm., the average growth increment being 3 to 5 gm. for this amount. This corresponds closely to Sherman's vitamin A unit.

The rate of fading of carotene dissolved in different solvents was determined by comparing 0.017% solutions with a standard $K_2Cr_2O_7$ solution colorimetrically. Carotene fades very rapidly in ethyl oleate, less in ethyl laurate. The color is fairly stable in ethyl oleate with hydroquinone (0.1%), and disappears most slowly in cottonseed oil. If a synthetic solvent is to be used, ethyl laurate protected with hydroquinone seems the logical choice. Whether the fading in solution is comparable to the fading of the crystals, is uncertain, but in either case, the physiological activity is lost.

Carotene is a pro-oxidant. 0.06% dissolved in an autoxidizable oil shortens its induction period by one-half. It is possible that this property is closely allied to its function as vitamin A.

5249

Determination of Butyl and Ethyl Alcohols in Fermentation Mixtures.

C. H. WERKMAN AND O. L. OSBURN.

From the Department of Bacteriology, Iowa State College, Ames, Iowa.

An accurate and rapid method for the quantitative determination of alcohols in fermentation mixtures would prove of value to the zymologist engaged in studies of the chemism of microbial action. A method which has been found very satisfactory is based upon the oxidation of the alcohols in the neutral volatile distillate by potassium dichromate and phosphoric acid. Ethyl alcohol yields acetic acid and butyl alcohol yields a mixture of butyric and acetic. The acids are quantitatively determined by the partition method.[1] The method is presented in its present form for the quantitative determination of unknown mixtures of butyl and ethyl alcohols, such as are found in the "butyl-acetone" fermentation. This fermentation has extensive industrial use in the production of butyl alcohol and acetone.

Fifty cc. of the alcohol mixture are pipetted into a 200 cc. balloon flask containing 10 gm. of potassium dichromate and 25 cc. of c.p. 85% phosphoric acid. Three or 4 small pieces of porcelain are

[1] Werkman, C. H., *Ind. Eng. Chem.* (analytical edition), 1930, **2**, 302.

added to insure smooth boiling and the flask is connected to an efficient reflux condenser. The flask is then heated at such a rate that the mixture is brought to a boil in one and one-half minutes. Gentle boiling is maintained for 3 minutes, making the total time of heating between 4 and 5 minutes. It is advisable to wash down the condenser tube occasionally by adding 5 cc. of water at the top. The volume of liquid in the flask should never exceed 100 cc.

After oxidation is complete the flask is quickly connected to a Liebig condenser and distilled until the mixture in the flask begins to foam. The heat is then lowered until foaming just continues. The heating is then continued for 2 or 3 minutes. If this procedure is carried out carefully all of the volatile acid is carried over but none of the phosphoric. The mixture has no tendency to bump or spatter.

The distillate is made up to 100 cc. and the total acid determined by titrating 25 cc. The result should be expressed in cc. of 0.1 N acid.

Thirty cc. are then partitioned with iso-propyl ether as described by Werkman.[1]

Under these conditions ethyl alcohol is quantitatively converted to acetic acid and butyl alcohol to a mixture containing 90% butyric acid and 10% acetic acid. This ratio of butyric to acetic acid was determined by oxidizing 5 butyl alcohol solutions varying in concentration from 50 mg. of alcohol to 600 mg. per 50 cc. The percentages of butyl alcohol found were 90, 90.2, 90, 90.5, and 91.

The calculations are most easily made in terms of cc. of 0.1 normal solutions.

From Werkman[1] may be read the per cent of butyric acid. This per cent divided by 0.9 and multiplied by the cc. of 0.1 N acid in the distillate gives the total cc. of 0.1 N acid derived from the butyl alcohol and designates the cc. of 0.1 N butyl alcohol in the alcohol mixture.

The difference between the total acid and that representing butyl alcohol gives the cc. of 0.1 N acid representing the ethyl alcohol. An example will make the meaning clear.

Assume that the distillate contains exactly 100 cc. of 0.1 N acid. The partition constant is 16.5. Reference to the nomogram[1] shows that the mixture contains 60% butyric and 40% acetic acid. Then $0.60 \times 100/0.9 = 66.6$ cc. of 0.1 N acid $= 66.6$ cc. of 0.1 N butyl alcohol. $100 - 66.6 = 33.4$ cc. of 0.1 N ethyl alcohol.

The method was applied to 15 known alcohol mixtures. The maximum error was 3.5% for ethyl alcohol and 3.0% for butyl alcohol.

5250

The Healing of Late Rickets Coincident with Low Serum Phosphate.

GENEVIEVE STEARNS AND JULIAN D. BOYD.

From the Department of Pediatrics, College of Medicine, State University of Iowa.

The calcium and phosphorus metabolism of two white girls, 12 and 13 years old, with clinical, roentgenological and chemical evidence of rickets was studied over a period of several months. During this time there was definite roentgenological evidence of healing of the ricketic lesions; the amounts of calcium and phosphorus retained by each child were ample for bone formation; yet the serum inorganic phosphorus of each child remained consistently low, ranging from 2 to 3.2 mg. %. The healing was not due to seasonal influence for it occurred during the late winter months.

5251

Lipids Associated with Proteins Under Normal and Pathological Conditions.

MARY E. TURNER. (Introduced by R. B. Gibson.)

From the Department of Biochemistry, University of Iowa.

The literature contains scattered references to the attraction of proteins for lipids. However, there are no statements in regard to any variations between normal and pathological conditions. Also no definite conclusions have been drawn as to the character of the attraction, that is, whether it is a physical or chemical union.

The problem was undertaken for a twofold purpose. It was hoped that an insight into the relationships between the various lipids and proteins might offer a method for differential diagnosis should there be characteristic variations in the amount of lipid associated with the albumin and globulin fractions. It was also thought that a more comprehensive study might throw some light on the type of association.

Blood plasma and edema fluids from cases having a possible lipemia were investigated; among these were cases of diabetes, nephritis, cirrhosis of the liver, carcinoma, and cardiac failure. Normal blood was taken for comparison. Fatty acids, lecithin,

cholesterol (free and ester) and iodine numbers were determined in the extracted albumin and globulin fractions as well as in the whole fluid.

The proteins were removed in their respective fractions by means of ammonium sulfate, heat coagulated at the isoelectric point, and washed free from the salt. The fatty acids were determined by Bloor's oxidative method, the lecithin by oxidation and formation of the blue ammonium phosphomolybdate, the cholesterol by a modification of the Okey oxidative method, and the iodine numbers by Gibson's micro method.

Comparisons were made between the total lipid content and the lipid associated with the proteins, also the distribution of the latter between albumin and globulin was determined. Since the protein ratios are not consistent and the amounts of globulin and albumin are not equal, calculations on the basis of mg. percent do not give the true relation between the lipids and the respective protein lipid fractions. However, on the basis of mg. per gram of protein the figures can be compared.

The variations between cases were not sufficient to offer a suitable means for clinical diagnosis. Increased lipid content of the plasma or fluid was reflected in respective increases in the protein fractions. The plasma globulins had the greatest attraction for fatty acids and lecithins while the cholesterol was fairly evenly distributed. Whether this is a physical or chemical union cannot be stated as yet. In the edema fluids, the relative amounts of lecithin and cholesterol associated with the globulin fraction were much greater than in the case of the plasma.

5252

Reproduction and Lactation on Simplified Diets.

P. D. WILKINSON AND V. E. NELSON.

From the Laboratory of Physiological Chemistry, Iowa State College, Ames.

Guest, Nelson, Parks, and Fulmer[1] showed that on certain synthetic diets containing various grains as the sole source of vitamins B and G growth and reproduction in the rat were normal but mammary function was decidedly abnormal. Daniels and Hutton[2]

[1] Guest, A. E., Nelson, V. E., Parks, T. B., and Fulmer, E. I., *Am. J. Physiol.*, 1926, **76**, 325.

[2] Daniels, A. L., and Hutton, M. K., *J. Biol. Chem.*, 1925, **63**, 143.

showed that successful reproduction and lactation may be obtained on a milk and soy bean diet which they attributed to the peculiar inorganic complex present in the soy bean. This work was undertaken to determine the effect of different levels of soy bean as the sole source of vitamins B and G on reproduction and lactation and the effect of various animal organs on the above functions.

Rats grow at a normal rate with 10, 20, 40, and 73.3% of either Manchu, Sable, or Virginia soy beans as the sole source of vitamins B and G. Reproduction was normal on the lower levels of the 3 different soy beans but on the higher levels reproduction slowed down. Satisfactory lactation was not obtained on any level of the 3 beans investigated.

Lung and spleen from hogs and beef thymus did not improve the lactation of rats on 15% of Virginia soy beans as the sole source of vitamins B and G. Hog pancreas and pancreas, brain, and heart from cattle improved somewhat the lactation observed on 15% of Virginia soy bean. Very marked improvement in lactation resulted on supplementing the 15% Virginia soy bean ration with liver from hogs and cattle and with hog kidney. The young on the liver and kidney showed a superior rate of growth. The lactating factor was destroyed in liver at 120°C. Ether extract of liver shows little or no lactating properties but the residue does.

Six to 10 females and 3 to 4 males were employed in each experiment on soy beans. Four to 10 pregnant females were used in the experiments on various animal organs.

5253

Studies on the Lactic Acid Content of Blood from Normal and Denervated Muscle.*

H. M. HINES, C. E. LEESE AND G. C. KNOWLTON.

From the Department of Physiology and the Child Welfare Station, State University of Iowa.

These experiments were undertaken to determine whether skeletal muscle deprived of its nerve supply and undergoing the process of atrophy exhibited a lactic acid metabolism similar to that of normal intact muscle. A comparison was made of the lactic acid content of blood entering and leaving a denervated limb with that from the

* Aided by a grant from the Laura Spelman Memorial Fund.

opposite normal limb under the conditions of rest, exercise and adrenalin administration. The studies were made on 5 dogs 2 to 3 weeks after denervation. Simultaneous drawings of blood were made from the femoral artery and femoral veins of dogs under amytal or local procaine anesthesia. The lactic acid was determined in duplicate according to the method of Friedemann, Cotonio and Shaffer.[1] Blood was drawn before and one hour after the subcutaneous injection of 1/5 mg. of adrenalin per kilo of body weight. The exercise was induced by the application of induction shocks at the rate of 2 per second to the muscle groups with needle electrodes. The legs were weighted with a light load to restrict the extent of movement. The exercise was continued for a period of 20 minutes and was in progress at the time the blood was drawn.

TABLE I.
Mg. of Lactic Acid per 100 cc. Blood.

	Arterial	Normal Venous	Venous Denervated
Resting	16.6	17.1	17.1
Exercise	17.5	15.1	17.1
After Adrenalin	37.1	37.6	37.2

A summary of the average results appears in Table I. They indicate that the lactic acid metabolism of skeletal muscle undergoing atrophy of denervation is essentially the same as that of normal muscle as far as can be determined by analysis of blood. The difference between the oxygen content of arterial and venous blood from the exercising limb was more than double the difference noted in the blood from the resting limb. These differences are the only index we have as to the degree of exercise. It is to be noted that the venous blood from the exercising limbs had a lower lactic acid content than the arterial blood. This difference is more pronounced in the normal than in the denervated limb. These findings on the lactic acid content of blood during exercise were not anticipated in view of the studies of Hill, Long and Lupton.[2] It is suggested that our results may be due to an improvement in the circulation of the muscle. The stimulus and exercise may have caused a greater opening of the capillary bed and an improvement in the oxygen supply to the muscle as a whole.

[1] Friedemann, T. E., Cotonio, M., and Shaffer, P. A., *J. Biol. Chem.*, 1927, **73**, 335.

[2] Hill, A. V., Long, C. N. H., and Lupton, H., *Proc. Roy. Soc. B.*, 1924, **94**, 438; **97**, 155.

5254

Effect of Fasting Upon the Activity of the Intact Skeletal Muscle of the Rat.*

CHESTER E. LEESE, HARRY M. HINES AND DOROTHY P. JORDAN.

From the Department of Physiology and the Child Welfare Station, State University of Iowa.

The work of previous investigators has shown that fasting and undernutrition are usually accompanied by a diminished ability to perform muscular work and an increased susceptibility to fatigue. A few instances are recorded in which an individual's strength was increased for a short time during a fast and in which isolated muscles from fasting animals exhibited a diminished rate of fatigue.

These experiments were planned to determine the effect of fasting upon the rate of fatigue and the work done by the intact gastrocnemius muscle of the rat. Glycogen studies were made on the tissues of the fatigued control animals in order to determine whether a relationship existed between muscular activity and the amount of carbohydrate stores. The animals were kept under light amytal anesthesia and the muscle activated by supermaximal induction shocks at the rate of 2 to 3 per second. A lever, weighted with a load equal to one-half of the weight of the animal, was attached to the foot. The femur was held in a rigid position by a special clamp. It was found that muscles with their natural attachments performed much more work than when the cut tendon was employed as a point of attachment. The muscle was considered to be in a condition of complete fatigue when the application of a supermaximal stimulus failed to cause a perceptible movement of the lever.

TABLE I.

Fast in hours	KgM work per gm. muscle	Total No. stimuli to fatigue	% Glycogen in fatigued muscle	% Glycogen in control muscle	% Glycogen in liver (fatigued)	% Glycogen in liver (control)
0	2.8	67,420	.125	.538	.164	2.185
24	2.7	71,306	.078	.370	.067	.172
48	2.8	67,486	.056	.375	.063	.078
80	3.3	95,000	.090	.355	.072	.254
120	2.0	68,676	.109	.321	.046	.345
144	3.1	116,280	.131	.355	.084	.339

Table I summarizes the average results obtained from a study of 50 rats and an equal number of controls. The muscle glycogen was

* Aided by a grant from the Laura Spelman Memorial Fund.

materially reduced by a 24-hour period of fasting and longer periods did not cause any greater degree of reduction. The lowest valves for liver glycogen were noted at the end of the 48-hour periods. In the longer periods the liver glycogen was increased over the earlier low valves. Fatigue occurred when appreciable quantities of glycogen were present in liver and muscle. It was found that the amount of work done by the muscle and the number of stimuli required to produce fatigue was independent of the initial and of the terminal glycogen content of liver and muscle. Considerable variation was noted in the number of stimuli required to produce fatigue in animals of the same group. It was found that the intact muscle from animals fasted for periods up to 144 hours in duration did as much work and required as many stimuli to produce fatigue as the muscle of well fed animals.

Southern Section.

Tulane University School of Medicine, November 20, 1930.

5255

Observation on the Motility of the Gastro-Intestinal Tract of Starving Rats.

LEON J. MENVILLE, J. N. ANÉ AND S. N. BLACKBERG.
(Introduced by W. H. Harris.)

From the Department of Medicine, Tulane University, and the Department of Pharmacology, Columbia University.

Many reports have been made on the contraction of the stomach due to hunger, in man and animals. In certain instances the x-ray was used[1] and in others, mechanical contrivances were utilized to record the results. The radiological studies have included the emptying time of the stomach due to hunger. Ivy and Fauley[2] state that hunger or fasting causes the stomach to empty faster in both dog and man.

The experiments herein reported were made on normal, healthy rats that were gradually deprived of food over a period of 3 weeks, with no restriction as to the intake of water. This series consisted of 5 rats, averaging 342 gm. in weight. They were at first made to fast for a period of 24 hours and subsequently each rat was fed 2 gm. of normal food a day for a period of 21 days. Two of these rats died, one on the tenth and the other on the fifteenth day of the experiment. All of the rats lost weight, the average weekly loss in weight per rat was 54 gm.

The remaining rats of this series on the twenty-first day of starvation were fasted and made to abstain from water for 24 hours and then fed a 10 gm. mixture of 3 parts of buttermilk and one part of barium sulphate and permitted to eat for 20 minutes and then immediately fluoroscoped to ascertain if the stomach was filled. The fluoroscopic observations were continued every 15 minutes until the

[1] Rogers, F. T., and Martin, C. L., *Am. J. Phys.*, 1926, **76**, 349.

[2] Ivy, A. C., and Fauley, G. B., *Am. J. Phys.*, 1929-30, **91**, 206.

stomach and small intestines were found empty. The examination of the colon was made at greater intervals of time.

Three normal rats were fed the same food over the same time, but the quantity of food per day was 14 gm. per rat which is considered an average normal amount.[3] On the twenty-first day they also were made to fast and abstain from water for 24 hours, when they were given the same mixture as the other rats and examined in a similar manner by means of the x-rays.

In every one of the 3 starving rats a marked hypermotility was observed of the entire gastro-intestinal tract.

TABLE I. *Averages.*

Food	No. Rats	Wt. gm. 9/9/30	Wt. gm. 10/1	Wt. gm. Lost	Ate gm.	Cecum Ap. time	Stom. Emp. Time	Sm. I. Emp. Time	Col. Emp. Time	Rations per day per rat. 9/9 to 10/1
Starving	3	327	243	84	9.6	1:27	3:29	4:32	45 h.	2 gm.
Normal	3	240	249.2	0	8.2	3:10	7:24	9:49	70 h.	14 gm.

Table I represents the results obtained in these experiments. It will be noted that the rats of both series ate nearly the same amount of buttermilk and barium sulphate and that the passage of this substance through the entire gastro-intestinal tract in the starving rats was very much faster than in the control rats.

5256

Potassium Content of Human Cardiac Muscle.

L. C. SCOTT. (Introduced by Henry Laurens.)

From the Department of Tropical Medicine, Tulane University.

The investigations of Ringer,[1] Howell,[2] and Zwaardemaker[3] and their coworkers offer the strongest evidence that the element potassium is an indispensable factor in cardiac activity. This conclusion is derived from experimental work on hearts of the lower animals, as a rule amphibians. Zwaardemaker alone has endeavored to adapt his findings to human heart function, in that he has

[3] Donaldson, "The Rat".

[1] Ringer, S., *Am. J. Physiol.*, 1884, **5**, 247.

[2] Howell and Duke, W. W., *Am. J. Physiol.*, 1928, **21**, 51.

[3] Zwaardemaker, H., *Arch. f. d. ges. Physiol.*, 1924, **205**, 20.

theoretically estimated the kinetic energy developed by the emanation of the Beta particles of potassium.

Inasmuch as there are few reliable and accurate series of determinations of potassium in cardiac muscle to be found in the literature, Zwaardemaker's estimation must of necessity be approximate. It therefore seemed desirable that a relatively large number of potassium determinations be made using a method the results of which would be as nearly representative as it was possible to obtain them. The one of choice, an adaptation of the old platinum reduction procedure of Neubauer[4] appeared to be the best. A detailed description of the method with the few modifications will appear in the complete article.

Two series of determinations of potassium have been undertaken, the first comprising 25 hearts, which, because of later modifications in the analysis is not to be looked upon as being as representative as the second series of 30 determinations, of which 27 have so far been completed. However, there is considerable variation in the potassium content of the human heart and for the specimens obtained at that time the means of the first series at least may be regarded as approximations.

TABLE I.
Summary of 25 potassium determinations.

	Weight of heart in gm.	Dry subst. %	K. %	*K.E. in Erg. sec.
Mean	366	19.76	0.173	$9.51 \cdot 10^{-7}$
Probable error of mean	±2.122	±0.0253	±0.0054	±0.3051

*Kinetic energy developed by Beta particles of potassium atoms in 1 gm. muscle.

Zwaardemaker's estimate of the kinetic energy developed by the potassium in a human heart of 300 gm. is $4.10 \cdot 10^{-4}$ erg seconds. The above mean K.E. output for a heart of similar mass is only $2.85 \cdot 10^{-4}$ erg seconds.

The second series of potassium determinations, each with 3 or more very close checks, yields a mean content of 0.230% for potassium and a mean K.E. coefficient of $12.65 \cdot 10^{-7}$ erg seconds per gm. heart muscle. This is equivalent to an energy output derived from potassium of $3.79 \cdot 10^{-4}$ erg seconds for a heart having 300 gm. of muscle substance.

It seems probable that the second series when completed will yield results quite closely confirming Zwaardemaker's hypothetical kinetic energy output of potassium in the human adult heart.

[4] Neubauer, H., *Z. f. Analyt. Chem.*, 1900, **39**, 481.

5257

Effect of Feeding Raw Liver to Dogs Infected with Endamoeba Histolytica.

EDWIN S. KAGY AND ERNEST CARROLL FAUST.

From the Parasitology Laboratory, Department of Tropical Medicine, Tulane University.

Cleveland and Sanders[1] have stated that, when *Endamoeba histolytica* is grown *in vitro* on slants of liver infusion agar covered with fresh horse serum-saline 1-6 containing rice flour, a medium is provided in which the organism will carry on its entire life cycle. Encystation begins within 18 to 24 hours after the slant is inoculated and continues for 3 days or more, the actual time for encystation requiring 4 to 6 hours. They believe that "there is evidently something in the dehydrated media . . . . which makes encystment possible," whereas "the whole process of growth and encystation may be upset by the use of contaminated media," *i. e.,* by bacteria or yeasts in the culture media.

We have utilized the Cleveland-Sanders technic in cultivating our Tulane canine strain (Strain A) of *E. histolytica* and have been able to confirm their results as to the value of the liver infusion medium. In a representative case an extremely rich growth of trophozoites was obtained within 72 hours after the culture was started. Four days later no active forms were found, but there were cysts in great abundance. This culture was continued by inoculation with the cysts through 3 subcultures and then discontinued. In another case abundant growth of trophozoites was maintained for 9 days, when no trophozoites but large numbers of cysts were found. These latter were utilized through 5 subcultures, after which an overgrowth of Giardia and bacteria was apparently responsible for the death of the amebae.

Daily observations of our canine strain of *E. histolytica,* cultivated *in vivo* over a period of 10 months has failed to produce a single instance in which other than trophozoites were passed in the stool. This same statement also applies to our more recent Strain B (of canine origin) and Strain C (of human origin, in which only cysts were used for the first experimental inoculation). In all of our infected animals the stools were dysenteric or diarrheic in character or consisted of feces mixed with a large amount of mucus. We therefore had a series of infected dogs on which it was desirable to test *in vivo* the effect of liver substance on *E. histolytica.*

[1] Cleveland, L. R., and Sanders, E. P., *Arch. f. Protistenkde.,* 1930, **70**, 223.

Four animals on a balanced diet, carrying Strain A, were utilized for this experiment. In each case the dog had been infected for a week or more, showed active symptoms of dysentery, and was passing trophozoites of *E. histolytica* in the stool. A single portion of 250 gm. of fresh calf's liver was fed to each animal. In each case the bowel movements were markedly reduced within 24 hours and the stools became semi-formed, although considerable blood and mucus was incorporated with the feces. In 3 of the 4 animals cysts were present in the passed stools within one to 7 days and continued intermittently until the death of the animal (a few days to 3 weeks later). In each case proctoscopic examination over the period revealed active lesions, with trophozoites at the site of the lesion. In the one animal in which cysts were not formed *E. histolytica* trophozoites were present on the day following the liver meal but were not recovered subsequently. The animal died a week later of a profound bacteremia.

This feeding test suggests an important rôle which liver substance plays in providing a medium favorable for encystation of *Endamoeba histolytica* in its natural habitat in the bowel. The fraction of the liver responsible for this phenomenon is the subject of a further study. We believe, however, that the dehydration of the large bowel content is intimately associated with the problem of encystment.

5258

The Panama Strains of Human Strongyloides.

ERNEST CARROLL FAUST.

From the Gorgas Memorial Laboratory and from the Parasitology Laboratory, Department of Tropical Medicine, Tulane University.

The studies of Grassi,[1] Perroncito[2] and particularly those of Leuckhart[3] demonstrated that there were 2 different strains of the human Strongyloides, *S. stercoralis,* the one consisting entirely of a parasitic generation and the other having both a parasitic and a

[1] Grassi, G. B., *Rend. Inst. Lomb. sci. e. lett. Rendic.,* Milano, 2 ser., 1879, **12**, 228.

[2] Perroncito, E., *Atti Accad. Lincei,* Ser. 3, 1879-1880, **1**, 381.

[3] Leuckart, R., *Gesellsch. d. Wissensch. Math. phys. Klasse.* Leipzig, 1882, **34**, 85.

free-living generation in its life cycle. Leichtenstern[4] believed that the organism in warm climates utilized only the indirect development, while those in cooler regions utilized only direct development. This hypothesis, however, has been proved fallacious by the work of Darling,[5] Sandground,[6] Nishigori[7] and others.

Darling's series consisted of cultures from 23 infected persons in Panama, residents of the tropics for many years. Of these cultures 5 proved to be purely indirect in their development, 7 were direct only and 11 were combined strains.

During the summer of 1930 the writer studied 1662 native patients in the Santo Tomas Hospital, Panama, of whom 348 were positive for *Strongyloides stercoralis*. Of this series 276 were each examined 3 or more times. Twenty-nine of these (10.5%) were of clinical importance. From the entire series 27 cases of heavy infection, in which no hookworm infestation was present, were selected for culture. These cultures consisted of active larvae, which had been richly concentrated by the centrifuge method, planted on animal charcoal in sterile Petri dishes, and allowed to develop at room temperature (26-36°C.). Each culture was examined daily for a period of from 3 to 9 weeks. The results of this study indicate that 2 of the infections were purely of the indirect type, 13 were direct only, 7 were of a direct type in which dwarf filariform larvae were present in the freshly passed feces, and 5 were combination of these types. The term *hyperinfective strain* is proposed for the type in which dwarf (unfed) filariform larvae are passed in the feces, since there is considerable evidence that this is the strain which is responsible for hyperinfection of the host.

In 5 cases second cultures were made from the same individuals and in every instance these were similar to the original strains. In the indirect type filariform larvae of the free-living generation had developed as early as the sixth day of culture; by the fifteenth day the strain had completely died out. In the unmodified direct type the rhabditiform larvae of the parasitic generation grew rapidly, became ensheathed between the fourth and sixth day, and continued as active organisms up to 9 weeks, without exsheathing or metamorphosing. In the pure hyperinfective type the feces usually showed both unfed rhabditiform larvae of the parasitic generation

[4] Leichtenstern, O., *Deutsche Med. Wochenschr.*, 1898, **8**; *Arbeit. aus d. k. Gesundheitsamte*, 1905, **22**, 309.

[5] Darling, S. T., *J. Exp. Med.*, 1911, **14**, 1.

[6] Sandground, J. H., *Am. J. Hyg.*, 1926, **6**, 337.

[7] Nishigori, M., *Taiwan Igakkai Zasshi*, 1928, No. 276. (Japanese text with English abstract.)

and dwarf (unfed) filariform larvae of the same generation. In a few days the former always metamorphosed into the latter, without feeding, and in 6 to 12 days after the culture was started they had died out. In mixed types the indirect and the hyperinfective strains died out between the sixth and the fourteenth day and the active ensheathed rhabditiform larvae of the direct type survived.

This study confirms the work of Darling, Sandground and Nishigori. Furthermore, it substantiates Nishigori's observations on the correlation between filariform larvae in the freshly passed stool and chronic clinical cases in tropical countries. On the basis of the consistent metamorphosis of the larvae without feeding, from the rhabditiform to the filariform stage, frequently before evacuation of the diarrheic stool, there is adequate evidence to designate them as a separate strain, for which the term hyperinfective strain is proposed. These observations indicate that the viability of these respective strains is directly associated with the nourishment which is taken, primarily in the rhabditiform stage of the parasitic generation.

5259

Effect of Vital Stains on Cultures of Endamoeba Histolytica.

DONOVAN C. BROWNE. (Introduced by C. W. Duval.)

From the Department of Pathology and Bacteriology, Tulane University.

Because of the more or less elaborate fixation and staining methods used to differentiate the types of Amoeba, the clinician has found it impractical to carry this examination out in routine practice. The vital stains suggested themselves as a simple means by which the differentiated points might be accentuated and recognition made easier in the routine examination. References to vital staining of amoeba in the literature are very few.[1]

The technique of Sabine was found impractical because of the nature of the culture media or stool specimen. Aqueous solutions of the dyes were prepared from 1/10 to 2%. A capillary drop of the culture and stain were mixed thoroughly on the slide with an applicator. A cover glass is placed firmly over this, with a blotter or cloth to absorb the excess. If the cover glass is not moderately firm,

[1] Stitt, E. R., Practical Bacteriology, Blood Work and Parasitology. P. Blakiston, Son & Co., Philadelphia, 1927, 65.

difficulty may be experienced in focusing the oil emulsion. In 3 to 5 minutes the slides may be examined. The following dyes were used: Neutral red; Trypan blue; Janus green; Brilliant cresol blue; and Nile blue; and the effect noted on the motile forms of the amoeba. Staining reactions on the dying or dead organisms were not consistent.

Neutral red appears non toxic up to 1% solution—the motility being very little affected. The dyes were taken up quickly, and give a very clear differentiation between the endoplasm staining pink and the ectoplasm apparently untouched. Recognition under low power in feces as well as cultures, is very easy, the amoeba appearing as pink refractory bodies. Under oil emersion, the nucleus stands out very distinctly, and by careful focusing as the organism flows along, the chromatin granules appear as a thin line about the periphery, the center appearing more or less clear. As the motility is lost, the nucleus may become larger and the layers of chromatin appear irregular. In some instances the karyosome apparently becomes visible. Unfortunately, at this stage the karyosome seldom maintains its central position, and a definite differential point is lost. I have observed the active amoeba coli with neutral red in only a few instances, but there seems to be a distinct difference in the chromatin arrangement in the nucleus. The band appears much broader and irregular about the circumference and throughout the nucleus dots of chromatin may be irregularly distributed. These observations have not been in sufficient number to draw conclusions.

Brilliant cresol blue in our hands proved unsatisfactory. The active organisms do not take the dye well, and it seems more toxic and the motility is quickly lost.

Janus green stains the endoplasm a pale pink; the ectoplasm remaining unchanged. The nucleus stands out more clearly and because of the paleness of the stain is often more distinct than with the neutral red. This dye is tolerated very well up to 1% solution, but is taken up very slowly, and many organisms remain unstained for long periods. The fact that Janus green stains pink is dependent upon the acidity of the culture media.

Trypan blue, although not as toxic as brilliant cresol blue, is not tolerated well above 0.5% solutions. The staining is unsatisfactory and not consistent. Endoplasm may be a pale blue color, with the ectoplasm unchanged. Nucleus is often well outlined, with the chromatin appearing black, but the contrast with the stained endoplasm does not allow it to stand out as well as with the pink staining.

Nile blue is tolerated well in the weaker solutions up to 0.5%. The endoplasm takes a pale blue color; the ectoplasm appears much paler, with a greenish hue. This latter point was not consistent, and when used in combination with neutral red does not have any particular advantage as a contrast stain.

Neutral red and Janus green are the most satisfactory dyes used, so far. The endoplasm and ectoplasm are more clearly differentiated and the fact that the nucleus with its chromatin arrangement is more visible may have some clinical value.

5260

Bacteriology of the Liver in Normal Dogs.*

I. M. GAGE. (Introduced by Alton Ochsner.)

From the Department of Surgery, Tulane University Medical School, New Orleans.

The presence of organisms in the tissue of normal, healthy animals has been a controversy for some time. However, investigators have definitely proved that the tissues of normal, healthy animals harbor organisms. That the occurrence of organisms in normal tissue in the experimental animal has been a source of incorrect observation and deduction in certain experimental problems has recently been shown by Ellis and Dragstedt.[1]

C. B. Hawn,[2] working with Holmes Jackson, first described a spore-bearing bacillus found in the liver of normal dogs. Walbach and Saiki[3] studied this bacillus, using 23 normal, healthy dogs, and found this gram-negative, spore-bearing bacillus in 21 of the 23 dogs. Berg, Zan, and Jobling[4] cultured the liver from 11 normal dogs and found this gram-negative, spore-bearing organism in 100% of their animals. Ellis and Dragstedt,[5] in their investigations on liver autolysis *in vivo,* encountered the same organism in almost 100% of their animals.

We studied the bacteriology of the contents of the stomach, the duodenum, and the liver in a series of normal, healthy dogs and the

* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

1 Ellis and Dragstedt, *Arch. Surg.,* 1930, **20**, 8.

2 Hawn, C. B., quoted by Wolbach and Saiki.

3 Wolbach and Saiki, *J. Am. Med. Assn.,* 1909, **21**.

4 Berg, Zan and Jobling, PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 433.

5 Ellis and Dragstedt, *Arch. Surg.,* 1930, **20**, 8.

bacteriology of the stomach and the liver in 4 normal, healthy puppies, 8 days old. All specimens for culture were obtained under strict aseptic conditions. The material obtained from the animals was cultured aerobically and anaerobically in digest meat broth at 37.5°C. The positive cultures were studied by smear methods stained with gram stain after 24 and 36 hours' incubation.

The *liver* was cultured aerobically and anaerobically from 40 dogs. There were positive cultures in 37 dogs, negative cultures in 3. Gram-negative, spore-bearing bacillus was found in 34 of the dogs, giving a positive culture in 92.5% and the presence of a gram-negative, spore-bearing bacillus in 85% of the cases. The remaining 6 cases showed the presence of gram-positive, gram-negative bacilli and gram-positive diplococci.

Contents of the *stomach* were cultured 16 times out of a series of 20 dogs. A positive culture was obtained in 15 instances, and a negative culture in 1 case. In 10 instances the presence of a gram-negative, spore-bearing bacillus was demonstrated. This gives a positive culture of the gastric contents in 93.7% of the animals and the presence of a gram-negative spore-bearing bacillus in 62.5%.

Contents of the *duodenum* were cultured in 19 out of the 20 dogs. Duodenal contents gave a positive culture in 17 cases, or 89.3%, negative cultures in 2 of the animals, and the demonstration of a gram-negative, spore-bearing organism in 5 cases, or 26.3%.

In 2 cases the bile was cultured aerobically and anaerobically and gave a positive culture in both instances, the organism being a gram-positive diplococcus in both.

Culture of the portal vein blood from 5 dogs gave 3 negative cultures and 2 positive cultures. Both positive cultures showed a gram-positive diplococcus.

The culture of the stomach of the 4 puppies gave positive cultures in 100%, and smear from the culture showed the appearance of a gram-negative, spore-bearing bacillus in each case. In 1 of the puppies, long hair was found in the stomach. As the puppy's hair was very short, it was definitely proved that the entrance of hair into the puppy's stomach was from the mother, obtained by the puppy while nursing at the mother's breast. Culture of the liver in the 4 puppies gave positive cultures in only 2 instances. The liver from 1 puppy showed a gram-positive diplococcus and the liver from the second puppy showed a gram-positive bacillus.

From the high percentage of positive cultures and the demonstration of the gram-negative, spore-bearing bacillus in the stomach and duodenum of the normal adult dogs and the stomach of the

puppies, it was evident that there was a constant source of supply of these organisms. Therefore, the following investigations were undertaken. The hair from 5 normal adult dogs and from 4 puppies was cultured aerobically and anaerobically in digest meat broth. Positive cultures were obtained in each case. The gram-negative, spore-bearing bacillus so frequently found in the liver was demonstrated in each culture. Scrapings obtained from the breasts of the nursing mother were cultured, and the same spore-bearing, gram-negative bacillus, as described above, was found.

We believe the source of the organisms found in normal, healthy dogs' liver is obtained from the hair of the animals, the organism gaining entrance into the stomach by way of the mouth, resulting from licking of the body, passing into the duodenum and most probably up through the common duct into the liver. In only 1 case was an attempt made to identify the organisms found in the liver. This was a large, gram-positive organism, and cultural characteristics proved this organism to be the *B. welchii.*

5261

A Method for Determining Basal Metabolism of Fishes.

MAZIE ADKINS. (Introduced by E. S. Hathaway.)

From the Department of Zoology, Tulane University.

Various methods used by other workers in metabolism experiments with fishes were considered inapplicable to studies in which physiological experimentation was to be extended over a considerable period of time. The constant flow method used by Hall[1] was modified for the present study. Hall measured the metabolism of many fishes over short periods of time. The present method is adapted for more detailed studies and provides for greater care of the experimental animals. Although Hall employed urethane to inhibit the movement of the fishes, Wieland (1915) states that the use of urethane causes the CO_2 threshold to be raised in the animals thus treated, and Winterstein (1914) concludes that O_2 consumption is decreased with the administration of urethane. Injection with urethane would have to be repeated after a 2-hour period, with consequent mechanical injury and metabolic disturbance to the fish; therefore Hall's method of controlling movement would not be applicable to protracted studies.

[1] Hall, F. G., *Am. J. Physiol.*, 1928, **88**, 212.

Twenty-eight fishes, 18 individuals of the large mouthed black bass, *Micropterus salmoides* (Lacepede), and 10 of the bluegill, *Lepomis incisor* (Mitchell), were used. Except in preliminary tests all experiments were carried out on fasting fishes, to obtain conditions more or less comparable with those prescribed for testing basal metabolism in humans. Twenty-four hours were allowed to elapse after the fishes were collected so that the intestine would be empty of fecal matter; each individual was then weighed, and placed in a fish jar in which it was to be tested (Fig. 1 B) for several hours before a series of determinations was begun.

Metabolic rate was measured in terms of cc. of oxygen consumed per kilo of body weight per hour. Oxygen determinations were made by the use of the Winkler method as described by Kemmerer, Bovard and Boorman (1924). According to these authors the regular Winkler method is regarded as satisfactory except when the water contains at least 0.1 part of nitrite nitrogen per million or considerable organic matter. It was considered that the small amount of organic matter that the fish, under the conditions of the test, would be discharging into the jar would be removed by the flow passing through the fish jar before the concentration became sufficient to affect oxygen determinations.

The set-up for the experiments is illustrated in Fig. 1. In the employment of constant flow a stream of water was allowed to pass through the fish jar. Water drawn through the sampling tube (C) would be representative of the inflow into the fish jar. The rate of flow was controlled by elevation for the outflow tube (D). A sample was taken from here simultaneously with that from (C). The difference in oxygen concentrations in these 2 samples represented the amount of oxygen being consumed by the fish.

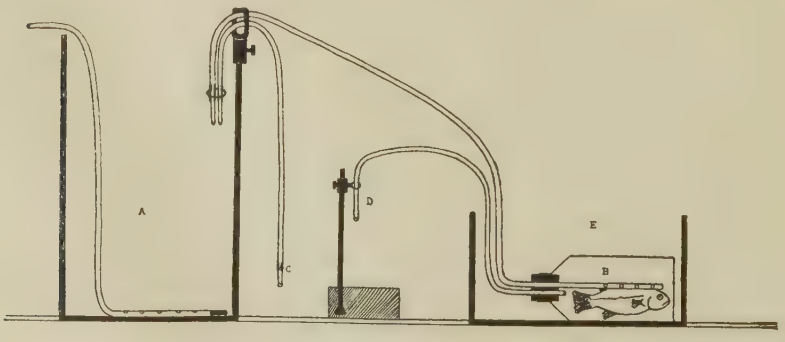

FIG. 1.
Diagram of apparatus used in metabolism experiments. (A) reservoir; (B) fish jar; (C) sampling tube from reservoir; (D) outflow tube from fish jar; (E) constant temperature bath.

In order that the water collected from the sampling tube represent the oxygen concentration of the water flowing into the fish jar the following conditions were observed. The reservoir was a 26 gallon tank, filled to overflowing from the laboratory tap, the end of the tube sealed so that all the inflow into the tank came through a number of comparatively small openings along the bottom of the tank. The upward currents thus produced circulation which prevented stratification or uneven distribution of oxygen. These precautions were necessary because early experiments showed wide hourly variations in oxygen content of the tap water. A mixing device in the fish jar similar to that installed in the reservoir provided that the inflow into the fish jar mixed freely with the water within. The rate of flow through the fish jar maintained in the jar at all times an abundant oxygen supply for normal metabolism. A constant rate of flow was maintained throughout each experiment.

The size of the fish jar was related to the size of the fish being tested. The fish was restricted to little more than respiratory movements of the fins and compensatory movement of the body. Constant temperature baths (E, Fig. 1) were 5-gallon crocks, the variation in temperature between inflow and outflow was at all times less than 0.5°C.

The glass fish jars with rubber stoppers were adapted in size to the fish being tested. One experimental animal, weighing 2100 gm. proved too large for convenient handling by this method. An aquarium was filled with water to within 3 inches of the top, and after the fish has been entered a layer of mineral oil, about an inch and a half in thickness, provided conditions approximating the air tightness of the fish jar. To guard against the fish getting into the oil a wooden lattice was secured just below the layer of oil. The rate of flow was regulated by running the water into a small container which could be raised and lowered at will. A constant level siphon provided for the maintenance of constant volume. During experimentation the sides of the aquarium were covered with an opaque screen.

In order to determine whether metabolic rate of fishes could be tested accurately by a method of intermittent flow the following procedure was devised. The apparatus of Figure 1 was used. Sample (1) was taken from the sampling tube simultaneously with Sample (2) from the fish jar. Then the flow of water into the jar was stopped. The determinations of (1)-(2) gave the oxygen consumption, with the use of constant flow. Fifteen minutes later

Sample (3) from the outflow tube of the fish jar was taken. The determinations of (2)-(3) gave the oxygen consumption with the use of intermittent flow.

The results of intermittent flow though themselves constant are noticeably lower than the results on the same individual using constant flow. This discrepancy might result from the following causes: 1. The oxygen tension of the medium in the fish jar varies with the use of intermittent flow. The fish, during the first 5 minutes of the test is extracting oxygen from a medium having an oxygen tension considerably different from the medium during the last 5 minutes of the test. According to Powers[2] oxygen consumption is affected by oxygen tension and carbon dioxide concentration of the media. 2. The carbon dioxide tension varies during the period of testing with intermittent flow. There is a resulting increase in hydrogen ion concentration which according to Powers might affect the oxygen consumption of the fish. 3. With the set-up regulated for simultaneous testing of continuous and intermittent flow, it was impractical to draw check samples for the intermittent flow determinations. The possibility of experimental error was thus increased.

The method of constant flow is therefore regarded as providing a greater degree of accuracy for the experiments at hand. Using constant flow 407 determinations were made on the 28 fishes tested. The time of testing an individual varied from 8 hours to 2 weeks, the number of determinations from 4 to 52. No attempt has yet been made to compile definite metabolic rates to be accepted as basal for fishes, even within the range of the species tested. The purpose was to develop a technique whereby fishes could be kept at a fairly constant rate of metabolism while undergoing physiological experimentation. Even the highly standardized procedure for humans admits considerable range of individual variation. The determination of the metabolic rate in fishes presents the added difficulties of control of a varying medium and the impossibility of completely controlling the movement of the fish. As yet little is known regarding the relation of age, sex, and physiological condition to respiratory metabolism of the fishes tested, and the purpose of the investigation was considered attained when results showed the fish to be metabolizing at a fairly constant rate. Duplicate determinations were taken and the results reported as a mean of the two. Deviations from the mean averaged less than 3%. When successive determinations of metabolic rate showed a varia-

[2] Powers, E. B., *J. Gen. Physiol.*, 1921, **4**, 305.

tion within ±15% it was considered that a degree of constancy had been reached comparable with that expected in basal metabolism for humans.

5262

Serum Calcium, Inorganic Phosphorus and Plasma Proteins in Cardiac Edema and After Diuresis.*

GEORGE HERRMANN.†

From the Medical Wards of Charity Hospital and the Tulane University School of Medicine.

Peters[1] and his coworkers have demonstrated the interrelationship of serum calcium, inorganic phosphorus and the total plasma proteins with albumin and globulin fractions combined. They have analyzed previous reports and their results and have evaluated the relative effects of protein and phosphorus on serum calcium in the absence of any true defect in calcium metabolism. The relation of the components in this series was defined by the equation $Ca = 0.255\ P + 0.566$ protein $+ 7$. An alignment chart was constructed to conform with this equation. The study of this blood chemical series in patients with edema of congestive heart failure was undertaken with the idea of establishing the presence or absence of any relationship between the series, but especially to determine the calcium level and the effectiveness or lack of the same on digitalization and other therapeutic measures.

The patients were all old white men and the normal levels for males established by Peters and his coworkers therefore are applied. The averages were: Total serum protein 6.93%; serum albumin 5.06%, and serum globulin 1.89%. The serum calcium normal is about 6 to 10 mgm. and the inorganic phosphorus is 3.5 to 15.6 mgm. per 100 cc. serum.

Only 4 of our cases are suitable for reporting now. All were arteriosclerotics, two, No. 1 and No. 3, with auricular fibrillation and two, No. 2 and No. 4, with normal sinus mechanism. Both of the latter died, while the fibrillators responded to digitalization with

* This study was made possible by a grant from the David Trautman Schwartz Research Fund.

† With the advice and cooperation of Dr. R. H. Turner and the expert technical assistance of Virginia Blanchard, Edna Seeliger Gouaux and Dorothy Drawe.

1 Peters, J. P., and Eiserson, Leo, *J. Biol. Chem.*, 1929, **84**, 155.

a diuresis and survived. One, No. 2, of the lost cases was definitely syphilitic with aortic regurgitation and hypertension and the other, No. 4, was probably also syphilitic with, however, only an aortitis. Both of the therapeutic failures, No. 2 and No. 4, had serum Ca (2) 11.3 mgm. and (4) 11.4 mgm. (—1.8% and +2.6% low respectively according to Peters' formula), while the total proteins were low, namely (2) 5.64% and 5.58%. The albumin fraction of each being greatly reduced (2) 3.02% and (4) 3.67%, while the globulins were slightly increased (2) 2.63% and (4) 1.9%.

In 2 cases of auricular fibrillation that diuresed even in the presence of considerable nephritis and improved, the Ca levels were 7.50 mgm. and 7.68 mgm., which were extremely low, (—40.2% and —39.5% lower than calculated normal according to Peters' formula). The inorganic Ps were exceedingly high, 7.90 and 4.18 mgm., probably due to the nephritis. These patients showed fairly normal total proteins, 6.22% and 9.48%, the serum albumins were decreased 4.22% and 3.36%, while the globulins were increased slightly, 2.00% in case 1, and greatly increased to 6.13% in case 3. With diuresis the Ca values rose to —19.4% below the normal of Peters' formula. These data warrant further and extensive study for corroboration.

5263

Interference Dissociation in Contrast to Reciprocating Rhythm.

GEORGE HERRMANN AND RICHARD ASHMAN.

From the Heart Station of the Charity Hospital and the Department of Medicine of Tulane University School of Medicine.

There is apparently no certain way of distinguishing between interference dissociation and reciprocating beats in the individual case, barring some accidental irregularity which affords a certain clue. There are, however, 2 criteria which serve to differentiate the conditions, (1) auricular regularity or irregularity and (2) P wave direction. If P waves are clearly upright in leads I and II or I, II, and III, it becomes certain that one is dealing with interference and not with reciprocation. If the auricles are regular, as is true in one of our cases, then the mechanism is in all probability interference. It would be a rare coincidence which would make the progressive prolongation of retrograde conduction time such as to make the

auricle regular; reciprocation therefore practically always presents an irregular contra-directional auricular activity.

Electrocardiograms are presented to call attention to the electrocardiographic differential diagnosis between "Interferenz Dissoziation" (Mobitz[1]) or Dissociation with Interference (Wenckebach and Winterberg[2]) and the entirely different mechanism of reciprocating beats for which it may be mistaken. The tracings show some conduction defect also, but this is not at all related to the unusual mechanism of interference which does not require the presence of defective conduction for its inception.

Interference dissociation will necessarily appear when there is free A-V conduction, complete V-A block, and an auricular rhythm which is slower than the ventricular. Under these conditions, those auricular impulses which follow the idioventricular beats by a great enough interval will reach the ventricle, since they encounter no absolutely refractory muscle in the conducting path. The ventricular beats they arouse will be premature. In our cases, as in the great majority of reported examples, the idioventricular pacemaker is supraventricular, *i. e.*, in the His bundle or A-V node. Consequently the transmitted auricular impulse must pass through that pacemaker and discharge it. The next idioventricular beat, therefore, follows this discharge after an interval equal to the typical idioventricular cycle length. It should be evident, at the same time, that there is no disturbance of auricular rhythm.

Thus the criteria which serve to set this mechanism apart from reciprocating beats are: (a) auricular regularity, or, in the presence of some irregularity, the auricular cycle during which the premature ventricular beats occur is not consistently longer than the others; and (b) P waves are of sinus, eudirectional, positive or upright form. As will be shown in our full report, there is, however, a *remote* possibility of both these criteria being present, especially in lead I, in case of reciprocating beats.

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[1] Mobitz, W., *Deutsch. Arch. f. Klin. Med.*, 1923, **141**, 257.

[2] Wenckebach, K. F., u. Winterberg, H., *Die unregelmessige Herztatigkeiten*, 1929, I.

New York Section.

New York Academy of Medicine, December 17, 1930.

5264

Electrocardiographic Changes in Pneumonia.

A. M. MASTER AND A. ROMANOFF.

From the Cardiographic Department and Medical Service of Dr. Leo Kessel, Mount Sinai Hospital, N. Y.

Twenty-one patients with lobar pneumonia, and 5 patients with broncho pneumonia were electrocardiographed *daily* to learn whether there was any graphic evidence of myocardial involvement during the disease. For the most part the patients were young adult males. No patient had received digitalis before admission or during his stay in the hospital. It is important to rule out this drug for it produces changes in the electrocardiogram.

Cohn and Jamieson[1] took one or 2 and occasionally more electrocardiograms in each of 56 patients who had received no digitalis. They used these patients as controls in their study of the action of digitalis in pneumonia. It is essential, however, to take daily records as some of the changes in the electrocardiogram are transient and disappear in a day or two. Cohn and Jamieson noted 2 cases with P-R and T changes, and 8 cases with T changes alone. What criteria were used for T-wave changes is not made clear, but we have only considered inversions or flattening of the T-wave, not mere change in size of a normal T-wave.

Early in pneumonia, and in very severely ill patients, T-wave inversions may appear; during the height of the illness or in beginning convalescence R-T deviations may be present; but during convalescence increased auriculoventricular conduction disturbances occur.

The *T-wave inversions* occurred in 4 individuals; 2 of the patients showed changes in leads II and III, the other 2 in leads I, II

[1] Cohn, A. E., and Jamieson, R. A., *J. Exp. Med.*, 1917, **25**, 15.

and III. The latter 2 patients died, one had a lobar and the other a broncho-pneumonia. In all 4 cases, the T-wave inversions disappeared in 24-48 hours. Flattening of the T-wave in lead I or II occurred 3 times and appeared of more significance when present in lead I. The *P-R intervals* were increased to 0.20 seconds in 7 cases; 0.22 seconds in one case; and 0.24 seconds in another. We feel that 0.20 seconds indicates an increase beyond normal as earlier in the disease the auriculoventricular conduction time was 0.16 seconds and usually less in every one of these 7 cases. The partial heart-block occurred in the stage of convalescence. Cohn and Jamieson reported one such case in which the P-R reached 0.21 seconds in the fourth week of convalescence. The *RST intervals* were abnormal in 20 cases. These changes were most common in leads I and II, and in all but 4 instances, were above the isoelectric level rather than below. In 4 of these patients the R-T transition intervals were markedly abnormal and similar to alternations in the electrocardiogram noted in acute coronary closure. Levine and Brown[2] and more recently Shearer[3] described one such case each in pneumonia. The slight or moderate changes in the R-T were definite. Either at the beginning or the end of the disease these intervals were normal. It is of interest that 19 out of 21 patients with lobar pneumonia showed R-T changes and only one in 5 of the broncho-pneumonia group.

Other findings were: a sinus arrhythmia 11 times; a change in the form of the P-wave in lead III, 3 times; a change in the T-wave in lead III, 15 times; a change in voltage of the QRS group, 4 times; a change in ventricular preponderance, 3 times; auricular fibrillation, once; "alternation" of the QRS complex, once; and a change in form of the QRS group, once.

The six patients who died all had tachycardia. T-wave and R-T changes were more common than in the patients who survived. On the other hand no increased P-R intervals were observed.

The electrocardiographic changes in pneumonia are very similar to those found in rheumatic fever and coronary artery disease. The increased P-R intervals and the T-wave inversions are similar to those found in the former illness, and the T-wave and R-T abnormalities are similar to the changes seen in patients with acute coronary artery closure.

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[2] Levine, S. A., and Brown, C. L., *Medicine*, 1929, **8**, 245.

[3] Shearer, Margery, *Am. Heart J.*, 1930, **5**, 801.

5265

Biochemical Studies of Human Semen.*
II. The Action of Semen on the Human Uterus.

RAPHAEL KURZROK AND CHARLES C. LIEB. (With the assistance of Sarah Ratner.)

From the Departments of Biochemistry, Pharmacology, and Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University.

In the treatment of human sterility, one of us (R. K.) has made many attempts to secure pregnancy by means of artificial insemination. Of the dozens of attempts, only 2 were probably successful. In a number of cases it was observed that when 0.5 cc. of semen was injected into the uterine cavity, the semen was promptly expelled, even though the patient was kept in an extreme Trendelenburg position. A similar quantity of Ringer's solution similarly injected was invariably retained. The patient always gave the same reaction, apparently independent of the phase of the menstrual cycle. These observations led to the following questions: What is the action of human semen upon the human uterus? Do 2 human uteri ever react differently to the same semen? Does one uterus react differently to the semens of 2 different individuals? The answers to these questions can be found in the following observations.

All material (uteri and semen) used for these experiments is of human origin. The uteri were obtained from the operating rooms of the Sloane Hospital, through the kindly cooperation of Dr. Benjamin P. Watson. Immediately after the removal of the uterus from the patient, adjacent strips were cut from it, and dropped into iced Ringer's solution, and placed in the refrigerator until required for an experiment. The strips were cut parallel to the fibers of the external muscular layer.

The semen specimens were obtained from private and clinic patients. Many were delivered directly into sterile jars; a few were condom specimens. The specimens were brought to the laboratory immediately after collection, and placed in the ice-box, at 5° C. The experiments were done as soon as possible after the collection of the semen; usually within 3 hours. The uterine strips were suspended in 100 cc. of warm, oxygenated Ringer's solution, and 1 cc. of the warm semen was added. In all cases, contraction of the uterus caused ascent of the lever.

* Mucin of the Cervix Uteri. I. R. Kurzrok and Edgar G. Miller, Jr., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 670.

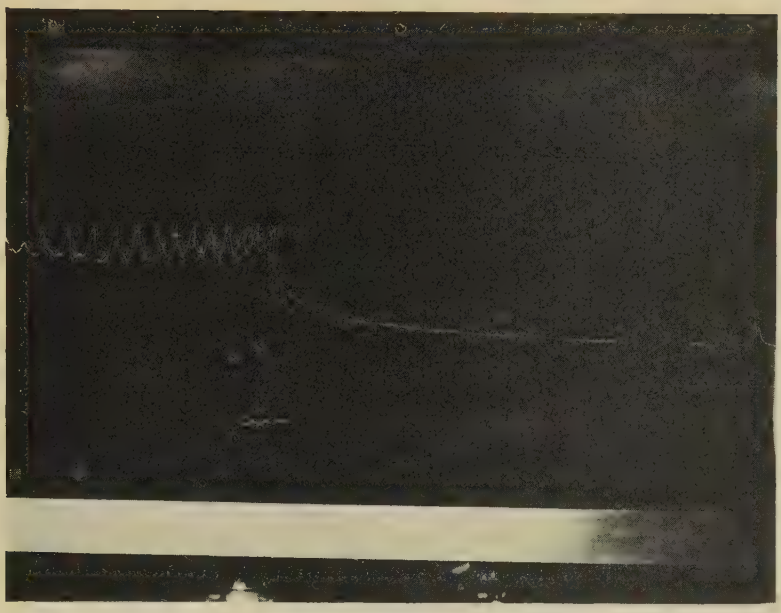


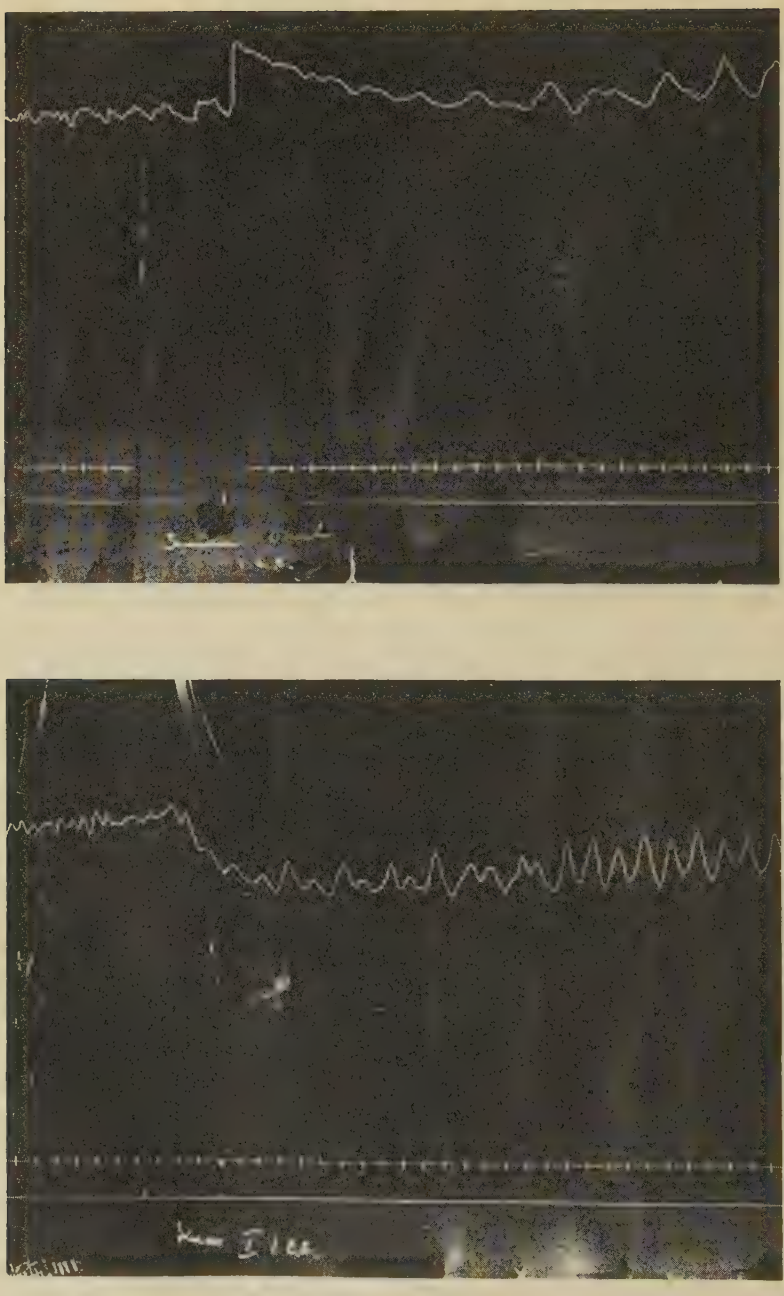
FIG. 1.
A segment of uterus (No. 81753) contracting in 100 cc. oxygenated Ringer's solution. At sequel, 1 cc. of semen was added to cylinder. Contraction-time in this and subsequent figures in minutes.

Fig. 1 shows one typical effect obtained by the addition of semen (No. X) to a contracting uterine strip (No. 81753). The result is a marked relaxation of the uterus, due to loss of tonus. There is marked inhibition of all contractions. This suppression of contraction lasts, on an average, 1 hour, when contractions reappear, develop gradually in strength, but remain in low tonus for several hours more.

Other experiments using different semen and uteri give, not depression but distinct contractions, with a marked rise in tonus, and more or less spasm, during which the individual contractions are extinguished.

Figs. 2 (a and b) show the action of 2 specimens of semen upon the same uterine strip (No. 270079). In Fig. 2a the semen (O.N.) produced a distinct contraction, with temporary spasm. Semen No. 1 caused decrease in strength of contraction, and marked loss of tonus on an adjacent strip of the uterus (Fig. 2b). Later, semen (O.N.) was added to this cylinder, and produced an effect comparable to Fig. 2a.

Fig. 3 is a tracing of 2 strips from 2 different uteri contracting in the same bath; the environment of the 2 strips is, therefore, identical. The addition of semen (No. 1) produced a definite contrac-



FIGS. 2a and 2b.
The graphs were made by 2 adjacent segments taken from the same uterus (No. 270079). In 2a semen (No. 0.N) caused contraction, while in 2b semen (No. 1) caused inhibition of the uterus.

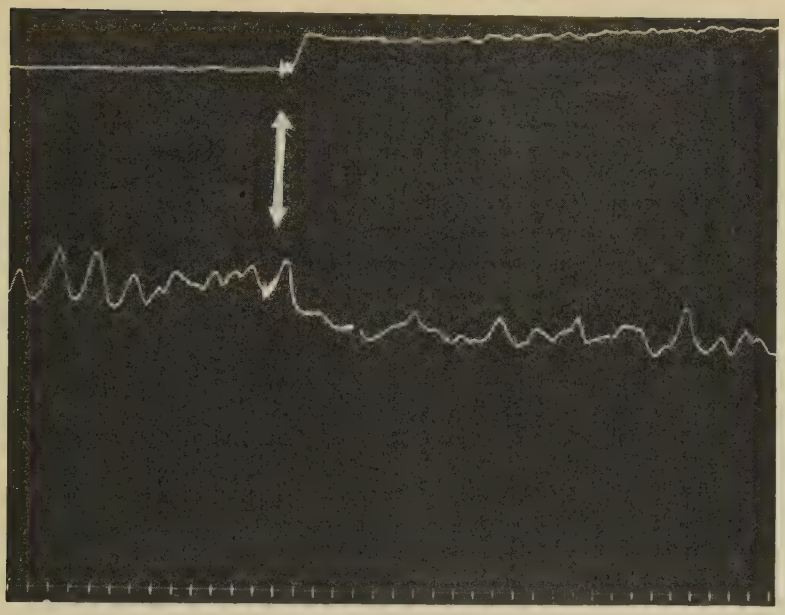


FIG. 3.
Upper tracing made by strip of uterus (No. 271451). Lower graph made by segment of uterus (No. 271839) both segments suspended in same chamber. At arrow, 1 cc. of semen (No. 1) was added.

tion of one uterus (No. 271451), and a well-defined relaxation in the other (No. 271839).

The age of the specimen of semen may influence its action on the uterus; thus a given semen may cause relaxation of a uterine strip, if applied within 3 or 4 hours after collection. If it is kept at 5°C. for 24 hours and then tested on another strip of the same uterus, it may cause a definite contraction. This reversal of action is not due to a change of the pH of the semen. Dr. Edgar G. Miller, Jr., was kind enough to dialize some specimens of semen, and we found that the active agents passed through the collodion membrane.

Spermatozoa are not essential to the reactions described in this paper.

We are indebted to Dr. Harold W. Dudley, of the National Institute of Medical Research, London, for supplying us with some spermine, which he prepared and found relatively non-toxic (personal communication).

Summary. The same uterus may react to one semen by contraction; to another by relaxation. The same semen may contract one uterus and relax another. From this we may draw the tentative conclusions that certain types of sterility are sometimes due to the

female, sometimes to the male. A study of the history of the patients from whom uterine strips were obtained throws an interesting light on our experiments. The uteri from the patients who give a history of successful pregnancy responded to fresh semen by relaxation, while uteri from women who gave a history of complete or long-standing sterility were always stimulated by semen. A tentative deduction is permissible: Uteri are of 2 kinds, receptive and rejective; semina are also of 2 kinds, stimulant and depressant. A large series of carefully selected cases will be studied, with the purpose of criticising these deductions.

We wish to thank Professor Benjamin P. Watson for putting at our disposal the large clinical facilities of the Sloane Hospital and Vanderbilt Clinic.

5266

Induction of Tetany in Rachitic Rats by Means of a Normal Diet.

A. F. HESS, M. WEINSTOCK, H. R. BENJAMIN AND J. GROSS.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In the study of experimental tetany, removal of the parathyroid glands has generally been resorted to. Following this procedure the calcium concentration of the blood promptly falls and the animal develops tremor or convulsions. Dogs have commonly been used and the thyroid as well as the parathyroids have been extirpated. It is evident that, from a pathogenetic standpoint, this procedure can not be compared with the mechanism of infantile tetany. Another method is to induce tetany by giving large amounts of the phosphates. This procedure likewise can not be considered satisfactory, as it does not reproduce or simulate conditions associated with the development of tetany in human beings. Since infantile tetany comes about almost invariably as a sequel to rickets, it is remarkable that investigators have not more often attempted to induce tetany as a complication of a previously existing rickets. Shohl[1] has clearly recognized the importance of bringing about tetany in rachitic animals and has reported a number of investigations in which this disorder was occasioned by giving phosphates to rachitic rats. More recently Hamilton[2] and his coworkers have induced tetany in rabbits which previously had been rendered rachitic.

[1] Shohl, A. T., and Brown, H. B., 1929, **84**, 501.

[2] Hamilton, B., Kajdi, L., Meeker, D., *J. Biol. Chem.*, 1930, **86**, 331.

Our object was to ascertain whether tetany could be induced in a rachitic animal by means of a ration which more nearly approached the dietary of the infant. The first step was to bring about rickets in young rats in the usual way by feeding them a high calcium, low phosphorus diet. The McCollum ration was used, which has a Ca:P ratio of about 4:1. This ration was fed for about 21 days with the induction of typical rickets. For the development of tetany the Sherman B normal diet was used. This is composed of one-third dry milk, two-thirds whole wheat, with the addition of NaCl; its Ca:P ratio is about 0.67:1 and it is stated to be adequate for growth and nutrition. After a period of 1, 2 or 4 days it was found that the calcium concentration of the blood of the rachitic rats had fallen from about 10 mg. to about 6 mg. per 100 cc., and in turn that the inorganic phosphate had risen from the rachitic level of about 3 mg. to 8 or 10 mg. Some of the animals showed definite tremor on etherization, which in some instances developed into convulsive seizures. The chemical change in the blood was noted even after this normal diet had been given for a period of only 8 hours; after this interval, although no fall in calcium may have resulted, there was a rise in the phosphate concentration. If this dietary was continued for a period of a week or 8 days the calcium rose to normal, the phosphate receding to its previous level more slowly. The animals did not lose any weight so that the manifestations could not be interpreted as being the result of starvation. Radiographs of the bones showed definite healing, even after an interval of but 2 days or less.

The same phenomena could be induced in rachitic rats by means of a ration which contained two-thirds dry milk and one-third whole wheat and in which the Ca:P ratio was about 1:1 or even on a ration of whole dry milk which had an excess of calcium over phosphorus (1.3:1). In order to ascertain whether the fall in calcium could be brought about by having a still greater excess of calcium, calcium lactate to the amount of 2.9% was added to the two-thirds milk ration; this addition rendered the calcium approximately equivalent to the calcium content of whole milk, making the ratio 1.7:1. Tetany was irregular on this diet. It was evident that in spite of the fact that the ration contained considerably more calcium than phosphorus, the calcium concentration in the blood rapidly fell below the normal level.

Experiments were next carried out to ascertain whether the changes were the result of an increase in absolute amount of the

phosphorus rather than in the ratio of Ca:P. 3.3 gm. of KH_2PO_4 was added to the McCollum diet, rendering the phosphorus equivalent to the content of calcium in the McCollum diet, and the ratio of Ca:P was made about 4:4. On this ration the calcium in the blood fell as with the previous rations. We next made up a diet similar to the McCollum ration with the exception that 2.36% less of $CaCO_3$ was incorporated; the standard amount of $CaCO_3$ is 3%. By this means a ratio of Ca:P of 1:1 was brought about and the ration did not contain any more phosphorus than the rickets-producing McCollum ration (0.302 gm.). Nevertheless the calcium concentration in the blood fell after a period of 2 to 4 days, and the phosphorus rose.

It was evident that the tetany did not result from giving a ration in which phosphorus was relatively high compared to calcium nor in which it was greater in absolute amount than in the McCollum ration. The sudden fall of calcium in the blood with the accompanying symptoms seemed rather to be the reaction to the sudden change of the Ca:P ratio as compared to the high ratio of (4:1) which characterized the rickets-producing diet. This sudden change, associated with healing of the rickets, evidently so disturbed the Ca:P balance in the body that the calcium concentration in the blood could no longer maintain its level and hyper-irritability of the nervous system followed.

5267

Fate of Orally Administered Specific Polysaccharide of Pneumococcus.

VICTOR ROSS.*

From the Bureau of Laboratories, Department of Health, New York City, and the Biochemical Department of the College of Physicians and Surgeons, Columbia University.

The author reported that, following the ingestion of Type I pneumococcus polysaccharide, white rats possess an increased resistance to the homologous organism.[1] It consequently became of interest

* Wm. J. Gies Fellow in Biological Chemistry, Columbia University. The author desires to thank Mrs. Lawrance Harriman for kindly providing funds in aid of this work.

[1] Ross, Victor, PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 658.

to learn something concerning the fate of this material when thus administered. Furthermore, the promptness with which immunity appears[2] after feeding the pneumococcus, suggested the oral administration of dead pneumococci in the early stages of human pneumonia as a therapeutic measure. It therefore became of importance to know whether the contained soluble specific substance enters the circulation. If it does, such a procedure would seem to be contraindicated, since it has been shown that the specific polysaccharide inhibits the pneumococcidal action of a normal serum-leucocyte mixture.[3]

Since the carbohydrate confers immunity when taken by mouth it seems reasonable to suppose that it is absorbed from the intestinal tract. Its polysaccharide nature would suggest, on the other hand, that its absorption as such is improbable.

The blood serum was examined for the polysaccharide of Type I pneumococcus following oral administration. Interfering sediments obtained when some rat sera are mixed with horse serum caused considerable difficulty in deciding whether a given precipitate was indicative of the presence of minute amounts of the polysaccharide or not. Preliminary incubation of the animals' sera and of the immune horse serum largely avoided such interference. By adding known amounts of Type I polysaccharide to rat serum, it was found that the presence of 1 part in a million in serum can be detected with a fair degree of confidence in the result. In the experiments in which quantities up to 6 mg. were fed, we have been unable to find definite evidence that the polysaccharide is present in a concentration of 1 part per million in serum. It seems that if it enters the circulation at all, the polysaccharide is present in a smaller concentration than this. Insufficient examinations have been done to say definitely whether or not it can be found in the urine. More work will be done with blood and urine. However, it is of interest to note that a large proportion can be found in the feces.

Briefly, the method employed was as follows: The polysaccharide was extracted from the feces by means of HCl, the solution made alkaline, and the sediment removed by centrifugation. Following neutralization of the supernatant fluid, serial dilutions were made so that the dilution in the final tube was equal to approximately 1 part in 6 millions after adding the serum and on the assumption that all that was fed was present in the feces. Incubation took place in an ice box for 48 hours. The same amount of poly-

[2] Ross, Victor, *J. Exp. Med.*, 1930, **51**, 585.

[3] Sia, R. H. P., *J. Exp. Med.*, 1926, **43**, 633.

saccharide as that fed was added to the feces of a control rat, and the final precipitates in the tubes for this animal were given an arbitrary value of 10, while the precipitates in the corresponding tubes of the unknown were graded with these as standards. The figures for each animal were averaged and an average of these in turn was calculated. In several experiments, each of 5 or more rats, 75% to 85% of the quantities ingested have been detected in the feces. However, the errors are such as to yield a low value for the quantity present. Proper controls were set up. The averages for each of 7 rats in a typical experiment were, 99, 103, 110, 80, 86, 97 and 43%, giving a final average of 87%.

An experiment in which the soluble specific substance was injected subcutaneously showed that no detectable quantity was eliminated by the bowel. It follows from this that its appearance in the feces after feeding is not the result of absorption and resecretion, either with the bile or otherwise.

It has also been found that Type 2 polysaccharide is eliminated with the feces to the same extent as Type 1.

The recovered polysaccharide of Type 1 pneumococcus from the feces of one set of rats was fed to a new group of animals and these were thereby immunized.

In the case of mice, which unlike rats, have not been found to become more resistant to pneumococcus Type 1, following ingestion of the polysaccharide, the feces contain quantities comparable to those excreted by rats. Appreciable destruction of the carbohydrate thus does not take place in this animal either, and hence cannot be the explanation for the failure to protect mice with this substance.

5268

A Practical Method for Concentrating Chill Free Pneumococcus Antibodies from Sera Without Use of Salt Precipitations.

EDWIN J. BANZHAF AND A. J. KLEIN.

From the Bureau of Laboratories, Department of Health, New York City, and the Littauer Foundation.

Avery[1] and Felton[2] showed that pneumococcus antibodies were irregularly precipitated from the antisera by simple dilution of distilled water. Banzhaf[3] showed that by fractioning the antisera with

[1] Avery, O. T., *J. Exp. Med.*, 1915, **21**, 133.

[2] Felton, L. D., *Boston M. and S. J.*, 1924, **190**, 819.

[3] Banzhaf, E. J., PROC. SOC. EXP. BIOL. AND MED., 1925, **22**, 329.

sodium sulphate and also with ammonium sulphate the antibodies could be precipitated from the dialyzed globulin on dilution with distilled water. Felton,[4] using the sodium sulphate method to recover the antibodies from the dialyzed globulins, found that at this stage the chill producing substance could be eliminated by dilution. He added sufficient normal sodium sulphate to a point where most of the antibodies were held in solution (the amount depending upon the length of dialysis). He then corrected the reaction to pH 4.8, at which point antibodies are in solution and a considerable amount of inert proteins together with the chill producing substances are precipitated.

It occurred to us that if we diluted antisera to a point where there was a definite precipitate and corrected the reaction to about pH 5, the small amount of antibodies precipitated would become soluble and the inert protein together with the chill producing substances would precipitate. This we found did occur. We obtained the same results without dilution by dialyzing the antisera practically free from salts and then adding sufficient sodium chloride or sodium sulphate to have a normality of about 1/20 and correcting the reaction to pH 5-5.1.

These methods were reported by Dr. William H. Park at the Harben Lectures in London October 10th, 1930, and are as follows: The cold antisera containing 0.4% trikresol or 0.5% phenol are diluted with cold distilled water, one part serum to 3 parts of distilled water, a definite precipitate occurs. When corrected to pH 5-5.1 using gamma dinitrophenol 0.025% solution as indicator, precipitated material will almost completely dissolve, followed immediately by a reprecipitation of inert protein together with the chill producing substances. It is then kept at from 5°C. to 10°C. for 4 hours, then filtered clear through paper pulp and adjusted to pH 6.8 using para nitrophenol 0.1% solution as indicator. One-tenth per cent trikresol or phenol is added at this time and allowed to remain in a cool place over night as usually a fibrin like substance will be noticed next day. If present it is again passed through paper pulp and trial tubes set up to determine the amount of distilled water necessary to precipitate the antibodies. This rarely requires more than an equal volume of distilled water. The antibodies are allowed to settle over night in a cold room. The supernatant is then decanted and the precipitate placed in cold centrifuge cups and rapidly centrifuged. The centrifuged precipitate is roughly estimated in cubic centimeters and a like amount of one per cent sodium chloride containing 0.8%

[4] Felton, L. D., *J. Inf. Dis.*, 1925, **37**, 199, 309.

phenol is added and the precipitate dissolved. The volume measured and diluted with 0.5% salt solution containing 0.4% phenol to one tenth of the original serum used. The solution is kept in a cool place for at least 24 hours for any possible clouding or precipitation that may occur. It is then passed through paper pulp to clarify and one percent sodium chloride added to bring the salt content up to 1.5%. It is then filtered (Berkefeld) and tested for sterility and potency.

The method of concentrating the antisera without the primary dilution is as follows: Antiserum containing 0.4% trikresol is dialyzed in running tap water for 3 days and 0.2% of trikresol is added and sufficient sodium chloride to have the salt concentration 1/20 normal. It is then chilled to 5°C. after which the reaction is corrected to pH 5-5.1. The method described in the foregoing is now followed to conclusion.

As this work was finished the November *Journal of Immunology* was received. In it Doctor Kenneth Goodner reports on experiments on the concentration of antipneumococcus and antimeningococcic horse sera. His work and ours is somewhat similar. He stresses cold temperature and the necessity for determining by trial the amount of distilled water to be added to 5 cc. of the antisera to obtain the first cloud and then adding 8 cc. more. This he states will precipitate all the antibodies. His work indicates a purer antibody can be obtained than we report in this paper. He states, however, nothing about removing the chill producing substances which we believe to be in his product.

5269

A Practical Method for Concentrating Chill Free Pneumococcus Antibodies from Plasma Without Use of Salt Precipitations.

EDWIN J. BANZHAF AND A. J. KLEIN.

From the Bureau of Laboratories, Department of Health, New York City, and the Littauer Foundation.

Antipneumococcus plasma containing 0.4% trikresol is dialyzed in running tap water for 4 days. This will remove sufficient salts for the method to be described. The plasma and precipitate consisting mostly of fibrinogen, euglobulin and antibodies are removed from the dialyzing bags, sufficient sodium chloride or sodium sul-

phate is added to make the dialysate one-twentieth normal (50 cc. normal sodium chloride or sulphate to each liter) and placed in a cold room over night to lower the temperature to about 5°C. Two-tenths percent trikresol is then added and the mixture adjusted to pH 5 to 5.1, using gamma dinitrophenol 0.025% solution as indicator. The antibodies are soluble at this pH and salt content. This mixture is allowed to remain at about 8°C. for 4 hours, during this time the insoluble substance fibrin, fibrinogen, euglobulin and the chill producing substance will have flocculated and settled to about 1/20 of the fluid volume. At this cold temperature it is then filtered clear through paper pulp (filtration is very rapid, 20 liters within a half hour). The filtrate is adjusted to pH 6.8 using para nitrophenol 0.1% solution as indicator, and allowed to stand over night at cool temperature for further precipitation, principally fibrin like substance at this pH. It is then filtered through paper pulp and trial tubes set up to determine the amount of distilled water necessary to precipitate the antibodies. This rarely requires more than two and a half volumes of distilled water. The required water is added and the precipitate allowed to settle in cool room over night. The following morning the supernatant fluid is decanted and the remainder of the precipitate centrifuged. The centrifuged precipitate is roughly estimated in cubic centimeters and a like amount of one percent sodium chloride containing 0.8% phenol is added and the precipitate dissolved. The volume measured and diluted with 0.5% salt solution containing 0.4% phenol to one-tenth of the original plasma used. The solution is kept in a cool place for at least 24 hours for any clouding or precipitation that may occur. It is filtered through paper pulp to clarify and 1% sodium chloride added to bring the salt content up to 1.5%. It is then filtered (Berkefeld), tested for sterility and potency.

5270

A Modified Method for the Production of Antipneumococcus Serum in Horses.

EDWIN J. BANZHAF AND THEODORE J. CURPHEY.

From the Bureau of Laboratories, Department of Health, New York City, and the Simon Baruch Foundation for Research in Pneumonia.

The method of preparing antisera consisting of the use of formalinized sediment of 18-hour pneumococcus broth culture injected

intravenously into horses is one in general use. The comparison of the therapeutic value of such antisera on the basis of their relative mouse unit content has been generally adopted.

A previous communication by one of us[1] demonstrated the possibility of producing a potent antiserum by means of the injection of pneumococcus pleural exudate intravenously. This was based on the hypothesis that such an antigen was likely to contain products from both the invading organism and the reacting tissue of the host, and would therefore be more nearly comparable to that produced under natural conditions during the disease itself.

Knowing that animals under immunization respond with a greater concentration of antisubstances when soluble antigens are injected intramuscularly than when administered intravenously, it was decided to immunize horses by means of phenolized pneumococcus pleural exudate intramuscularly and formalinized sediment of 18 hour broth culture intravenously.

The sera of horses thus immunized have been tested by the mouse protection test, and fall in 2 groups, those of high unit value and those of low unit value. When such sera were compared by the method suggested by Goodner[2] with control antipneumococcus sera of high and low titre prepared by vaccine administered intravenously, it was found that their therapeutic value was disproportionate to their mouse unit content indicating the possible presence of added therapeutic substances resulting from the modified mode of immunization.

5271

The Titration of Pneumococcus "Exudate" Antisera.

THEODORE J. CURPHEY AND HERMAN B. BARUCH.

From the Simon Baruch Foundation for Research in Pneumonia and the Bureau of Laboratories, Department of Health, New York City.

In a previous communication[1] a method for the production of antipneumococcus serum by means of the immunization of horses with type specific pneumococcus pleural exudates was reported. Sera pre-

[1] Curphey, T. J., and Baruch, M. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 687.

[2] Goodner, K., *J. Exp. Med.*, 1928, **48**, 1.

[1] Curphey, T. J., and Baruch, H. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 687.

pared by this method were found to contain protective substances in about the same concentration as other sera prepared by intravenous immunization with pneumococcus vaccine, when tested in mice. However, in view of the fact that it was felt that the "exudate" antisera might contain certain anti-substances resulting from a possible antigenic effect of the cellular components of the inflammatory exudate (acting either alone, or in the nature of a haptene to the type specific pneumococcus protein radicle therein present) it seemed necessary to titrate such antisera under conditions in which both the bacterial and the cellular antigenic components would co-exist and be capable of some quantitative measurement. The method of producing an acute inflammatory lesion by intradermal injection of the pneumococcus as outlined by Zinsser[2] and later studied in detail by Goodner[3, 4] provided at the local site of inflammation both the bacterial and the cellular components in the course of an active infection of the skin, and therefore seemed suitable for measuring the potency of such "exudate" antisera.

Tests conducted with serum from a horse immunized with Type I pneumococcus exudate intravenously, and controlled with several sera from horses immunized with vaccine show that a larger number of rabbits are protected against a standard dose of Type I pneumococcus culture injected intradermally when they receive a given mouse unit dose of "exudate" antiserum, than when a similar dose of "vaccine" antiserum is administered at the same time after infection. Thus, of a group of 53 rabbits receiving a skin infection of about 200,000 organisms each, of 27 treated with 450 units of "exudate" antiserum, six hours after infection, 19 survived (70.4%) while of a similar group of 26 receiving 450 units of "vaccine" antiserum six hours after infection, only 9 (34.7%) survived.

These results while they represent the titration of a single "exudate" antiserum, seem striking enough to warrant the assumption that such an antiserum contains substances other than antibacterial antibodies, seeing that control antibacterial sera were far less able to affect the course of the local inflammatory lesion and to lead to as high a survival rate. Furthermore, work at present in progress with other samples of "exudate" antisera indicate that the difference observed above is not that of individual horse variation, lending support to the hypothesis that the cellular component of the "exudate"

[2] Zinsser, H., *Boston Med. and Surg. J.*, 1925, **192**, 1191.

[3] Goodner, K., *J. Exp. Med.*, 1928, **48**, 1.

[4] Goodner, K., *J. Exp. Med.*, 1928, **48**, 413.

antigen is an important contributory factor in the production of pneumococcus antisera.*

5272

A Simplified Method for Quantitative Tissue Culture in vitro.

MACHTELD SANO AND LAWRENCE W. SMITH. (Introduced by J. Ewing.)

From the Willard Parker Hospital, and the Department of Pathology, Cornell University Medical College.

Objects of Method: (1) to be able to observe the growth of tissue using high power microscopic objectives, as in the original cover slip method of Maximow; (2) to secure quantitative growth as has been made possible to a certain extent by the Carrel flask;* and (3) at the same time to retain the advantages of the cleverly devised Borel flask in which the bottom is detachable, so that the tissue can be fixed and stained without distortion, and thus retained as a permanent record of the tissue grown. The present method is a simplified adaptation and combination of all 3 of these techniques.

The simple apparatus consists of but 3 parts: (1) A glass ring 3 inches in diameter, 5-6 mm. in height, with parallel, flat, ground surfaces, 2-3 mm. in thickness. These are made from heavy pyrex tubing. (2) Two thin sheets of mica 3¼ inches in diameter. (3) An ordinary 4 inch petri dish.

Method: Absolute cleanliness of the glassware is essential We sterilize the glass ring within a petri dish, and sterilize the mica sheets separately in another petri dish for convenience in handling them. Working preferably in a sterile bacteriologic transfer room, to one edge of the sterilized glass ring is applied a rim of vaseline (1% paraffin) by means of a sterile wide mouthed pipette. One of the sterile mica sheets is placed upon it and pressed down. This is then turned upside down within the petri dish where it forms a chamber, the cover being the bottom, and the ring its wall. This is now ready to receive any medium, solid or liquid, and the tissue for culture. As the entire chamber is readily accessible by simply removing the cover of the petri dish, the fragments of tissue can be arranged

* We beg to express our gratitude to Dr. Wm. H. Park for his interest in this work and to Miss Lillian Gross for her technical assistance.

* Since presenting this paper we have learned that a somewhat similar, but more elaborate apparatus with a metal ring has been used by Carrel.

and grouped as desired. The area is large enough so that 50 or more such bits of tissue can be planted within the chamber, thus offering an opportunity for quantitative work, or for comparative studies by growing different tissues under identical conditions.

After the media and tissue have been added, the chamber is sealed

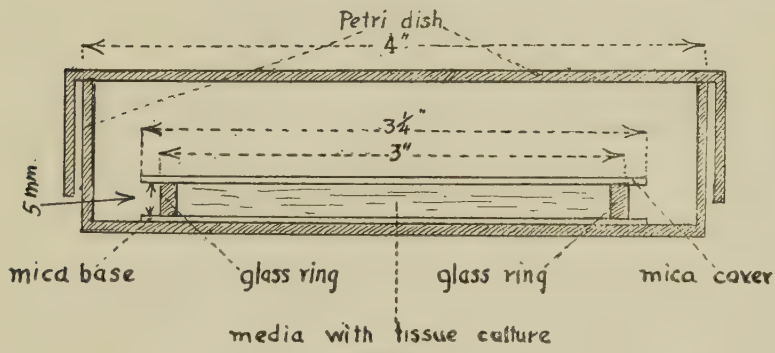


FIG. 1.

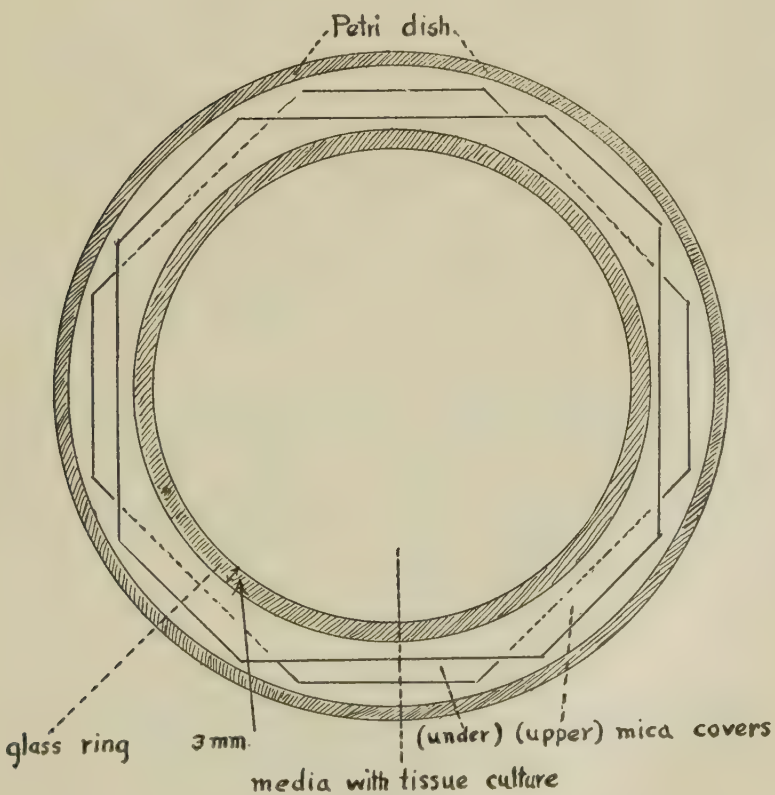


FIG. 2.

by the other mica sheet in the same manner as the first one. This culture chamber is kept in the petri dish for convenience in handling.

To examine the tissue the chamber is removed from the petri dish, inverted, placed under the microscope, and the growth observed under oil immersion. No special handling is required. To remove the products of metabolism for study, to wash tissue, or to renew its food supply, the mica cover is either partially elevated on one side or wholly removed, after placing the petri dish on a slight slant. The fluid, totalling 1-3 cc. is removed by a sterile, capillary pipette, the surface washed off by running in Tyrode's solution and removing it in the same way by pipetting. Pieces of dead tissue or living tissue for transfer can be removed with needle and lancet. Fresh food is then added and the chamber again closed by a fresh, sterile mica cover. When the culture is ready for disposal after it has grown sufficiently or has been used for inoculation, etc., the remaining material can be fixed and stained. It is only necessary to remove the bottom mica from the chamber, wash off the excess media and drop the mica into fixing fluid. The size of the mica sheet, 3¼ inches square, permits cutting into several smaller pieces and preparing various comparatively stained and fixed specimens for study, all from the same culture and grown under identical conditions. In this way, too, a permanent record is available for reference.

The chief value of the method appears to be in the opportunity afforded for quantitative studies of the products of cell metabolism. It can be applied to much larger volumes by simply increasing the size of the chamber. It offers a quantitative method of analytical approach to the whole field of cell physiology. The apparatus is easily cleaned and readily handled. It is applicable to either solid or liquid media.

We have successfully used the method for experimentally growing numerous types of tissue and for the multiplication of vaccine virus as an approach to some of the larger problems concerned with filtrable virus disease. We present the technique with the hope that it may save others many hours of discouraging labor, and thus contribute toward the ultimate solution of some of these as yet unsolved fundamental biologic problems.

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The Fetal Heart Rate in the Monkey (Macacus Rhesus).

CARL G. HARTMAN, R. R. SQUIER AND O. L. TINKLEPAUGH.
(Introduced by E. M. K. Geiling.)

From the Department of Embryology, Carnegie Institution, Washington, and the Primate Laboratory, Yale University.

In the early summer of 1929 the writers, in connection with a joint study of parturition and the behavior of the new-born monkey at the Carnegie rhesus colony, recorded the pulse of 11 pregnant females and in 6 subjects were able to record the fetal pulse. To these we add some observations of Dr. R. R. Squier, who auscultated 2 pregnant females in 1930 (Nos. 39 and 51).

The procedure was as follows: The animal was caught with a net and held by pinning her elbows behind her back. It is important to note that a vigorous animal may do considerable running before she is caught. The captured animal was laid on her side on a table and after she had become quiet the experimenters took turns with the stethoscope, checking each other. The maternal pulse was first taken and then the stethoscope was placed over the animal's abdomen and the spot found where the fetal pulse was discernible. This varied greatly and seems to be, in the monkey, unpredictable. Certainly, if the optimum point depends on the position of the fetus its situation might be expected to vary greatly. The characteristic double click of the fetal heart beat was heard well; likewise occasionally the placental bruit.

As will be seen by the accompanying table, the maternal pulse ranged from 160 to 240, the fetal from 100 to 180, at periods from 3 to 32 days prior to parturition and at a copulation age of 129 to 163 days. The exceptional cases will be discussed separately. For the human being, cow and dog, Clark[1] quotes the following figures:

	Fetus	Adult
Human	135 (5-9 mos.)	70
Cow	161	50
Dog	120-170	100

Williams gives 120 to 140 as the fetal heart rate in man in his *Obstetrics.*

These figures are sufficient to bear out the common opinion that the fetal is consistently faster than the maternal heart rate. Our figures are for the most part contrary to these, but the discrepancy

[1] Clark, *Comp. Physiol. of Heart*, 1927.

TABLE I. *Fetal and Maternal Heart Rates.*

Female No.	Days before delivery	Fetal Copl'n Age	Pulse Mother	Pulse Fetus	Days before delivery	Fetal Copl'n Age	Pulse Mother	Pulse Fetus	Days before delivery	Fetal Copl'n Age	Pulse Mother	Pulse Fetus	Days before delivery	Fetal Copl'n Age	Pulse Mother	Pulse Fetus
12	30	150	200	104	20	160	198	104								
			220	156			212	108								
							212	140								
34	3	152	160	139												
			200	140												
			240	146												
			230	118												
39	31	129	220	164	21	139	168	144	12	148	186	100	9	151	204	128
				160			180	160				144				122
			222	166							200	148			228	126
				172								160				128
				172												
40	32	138	214	172	22	148	220	110	10	160	228	116				
			212					112			200	130				
			216					140			200	176				
											224	180				
43	22	148	204		12	158	212	172								
							212	176								
69	6	147	208	114												
39	1	169	190	—	14	170	140	184	10	170	148	180				
					hours		144	188	hours							
51	5	163	188	144	12-16	168	168	180								
					hours		160	184								
								192								
							160	200								
								200								

is readily explained—the rapid pulse of the pregnant monkey is due to her excitement and vigorous exercise attending capture and subsequent struggles to escape. This is partly borne out by the pulse rate of a very tame pregnant female, *Macacus cynomolgus (Pithecus irus)* of the Yale Primate Laboratory. This animal after voluntarily climbing up on the experimenter's lap and while sitting there without restraint shows a pulse rate of 136. After strenuous romping with her cage mate the rate is increased to 188. On one occasion, in a large male with erect penis, the phallic pulse was 90.

The measurement of temperature offers a parallel case. We have made many readings of the rectal temperature which varies from 101° to 105.5° F. The younger females run about 104° on the average, the older ones 102.5°. This is certainly far above the normal temperature of the animal at rest. What this is we do not know.

It is clear that, in general, our results on the monkey are not transferable to other orders of mammals. It is seen that, whereas the fetal heart beat in other mammals is about double that of the mother, in the monkey the mother's pulse is double the heart rate of the fetus. The discrepancy is, therefore, fourfold.

An interesting aspect of the results is the increase of rate with successive readings made at the same sitting. This holds especially for the fetus. Partial asphyxia probably accounts for the accelerated heart.

We come now to consider the reversal in the relative heart rate of mother and fetus just before parturition. This consists principally of an acceleration of the fetal rate, though the maternal rate on the occasion of the auscultations involved was considerably slower than before.

As seen from the last items of the table, female No. 39 was auscultated on the 169th day of gestation (Oct. 30) but the fetal heart rate was missed. The next day the fetal heart rate was found to be 184 and 188, the maternal 140 and 144. Since it was first thought that there might be a mistake, the observation was repeated with 180 for the fetal, 148 for the maternal rate. Ten hours later the baby was born after one hour's labor. Immediately after birth the baby's heart rate was 200.

With these observations as a cue, female No. 51 was examined at 2:00 p. m. November 4, early in the 169th day of gestation. The table shows the result of counts by 2 of us; the relative heart rate was now the reverse of what it had been several days before, the fetal being much higher than that of the mother. On the basis of

the behavior of No. 39, birth of the second baby was sanguinely expected to occur promptly. At 9:00 a. m. the following morning (Nov. 5) the baby was found, still attached to the afterbirth, which the mother was handling and nibbling at but not eating. (It may be stated parenthetically that this same female failed to eat the placenta after giving birth the year previous.) The baby was dry, the placenta somewhat dry on the edge. Birth had therefore taken place some hours before, or 12 to 16 hours after the fetal heart rate had been taken. The baby's heart rate at 10:00 o'clock was 192.

The obstetrical question arises: does acceleration of the fetal heart rate presage impending labor? The answer must be left for further observation, easily carried out in the human. At present we know of no figures on the changes (or absence of changes) in the fetal heart rate taken for a week, let us say, before parturition.

5274

The Survival Period and Blood Pressures of Adrenalectomized Decerebrate Cats.

GERTRUDE B. SANDERS. (Introduced by H. C. Bazett.)

From the Department of Physiology, University of Pennsylvania.

In 1919 Bazett[1] described experiments on adrenalectomized cats showing that the time of survival after adrenal removal averaged 6 hours for decerebrate and 10 hours for lightly urethaned animals. Theoretically possible explanations, such as extra shock, hemorrhage, or pituitary injury in the case of the decerebrates, were advanced to explain this difference. It was emphasized that there was no immediate (within one hour) blood pressure fall even in animals surviving a few hours only. The present investigation to obtain experimental data to enable discrimination between the possible mechanisms for the more rapid death of the decerebrates, although so far unsuccessful in its aim, has disclosed facts pertinent to discussions in current literature.

Vincent and Thompson[2, 3] reported that adrenalectomy in decerebrate cats permitted survival for only ½ to 1 hour although control decerebrates lived "for several hours". Death in adrenalectomized

[1] Bazett, *J. Physiol.*, 1919, **53**, 320.

[2] Vincent and Thompson, *J. Physiol.*, 1928, **67**, proceedings.

[3] Vincent and Thompson, *Nature*, 1928, **122**, 998.

animals was caused by respiratory failure due, they believed, to removal of an hormone (normally carried by the lymphatics to the medulla from the adrenals) which they named "pneumin". In spite of the contradictory results of Florey, Szent-Gyorgi, and Florey,[4] Vincent and Thompson later reaffirmed their stand[5] although they increased the survival period to 1 to 2 hours after adrenalectomy They claimed that Florey *et al.* had based their criticism on too few experiments.

The adrenals in 30 cats, decerebrated by the Sherrington scoop method, have been removed at intervals varying from 1 to 18 hours after decerebration. Removal was by the lumbar route without asepsis. (In a few cases some antiseptic precautions were taken.) Death of adrenalectomized animals by respiratory failure was obtained only when: 1. the decerebration was unsatisfactory and itself a probable cause of death; 2. the trachea was occluded by mucus; 3. the animal had survived for at least several hours after adrenalectomy and was showing signs of circulatory changes. Only 3 of the cats survived less than 2 hours after adrenalectomy—½, ½, and 1 hour respectively. In these, death was preceded by obvious gastrointestinal disturbances (probably secondary to medullary hemorrhage) and was, therefore, not attributable to the absence of the adrenals. The average survival period (excluding these 3 but including animals subjected to various experimental procedures) was 8.5 hours after adrenalectomy. One-third of the animals lived 9 hours or more. The longest was 21.5 hours. Ten control decerebrates, with and without dummy adrenalectomies, averaged 18.5 hours of life although these included a number of animals which were killed when convenient.

The statement of Vincent and Thompson[5] that "there can be no doubt that if a cat be decerebrated and then, after 1 or 2 hours, the adrenal bodies be removed, symptoms of respiratory failure will not be long in appearing and the animal will be dead in an hour or so" is thus not confirmed.

Vincent and Thompson[6] claim that clamping of both the adrenal vein and the adrenal collateral circulation results in an enormous immediate fall in blood pressure. The low level persists for about 20 minutes or until the clamp is removed (if earlier than this). Recovery then usually takes place in another 20 minutes after which, if the clamps are still in place or if the adrenals have been removed,

[4] Florey, Szent-Gyorgi, and Florey, *J. Physiol.*, 1928, **67**, 343.

[5] Vincent and Thompson, *Nature*, 1929, **124**, 445.

[6] Vincent and Thompson, *Endocrinology*, 1930, **14**, 93.

the blood pressure falls again until the death of the animal in 1 to 2 hours. They compare this fall to that found by Bazett[1] although the latter never observed such an effect within one hour after adrenalectomy and the fall, when induced, continued until death.

Where blood pressure records were taken during adrenalectomies in the present investigation, it was found that not infrequently there was a small fall following removal of the gland. It was usually much less than 25 mm. Hg whereas Vincent and Thompson report 50 mm. Hg as an average value. In recovering from this drop when present the blood pressure usually reached a supernormal value before returning to its original level. It is possible that this is the same phenomenon as that described by Vincent and Thompson[6] although it is not so prominent and has by no means been invariably present.

A more direct experiment was also attempted. In a few cases all tissue was cut away from the adrenal gland except that immediately around the adrenal vein and artery at the hylus. Occlusion at this point then completely isolated the gland. That occlusion was complete was demonstrated by a typical adrenalin blood pressure rise when the thread was loosened after ½ to 1½ minutes. The experiment was done repeatedly on each animal. During occlusion there was at times a slight decrease in pulse pressure resulting in a small fall of mean pressure although the diastolic pressure remained constant. A similar fall in pulse pressure could, however, be obtained mechanically by occlusion of the vena cava. Since the circulation may be so easily affected during manipulation of the adrenal glands, the possibility of such a mechanical origin of the temporary fall of blood pressure can not be excluded. In view of this possibility, it should be noted that no fall was seen in these experiments when an occlusion series was finished and the adrenal tissue was completely ligated or the gland removed.

Based on both the direct and indirect experiments it is, therefore, problematical whether such a small and inconstant fall in blood pressure is significant.

5275

Residual Germicidal Action of Water and Plain Agar After Exposure to Ultraviolet Light.

FRED W. TANNER AND HELEN GEEN.

From the Department of Bacteriology, University of Illinois, Urbana.

Downes and Blunt[1] stated that the most active rays on microorganisms were blue, violet and ultraviolet, the red and orange were not entirely inactive. Ward and Lodge,[2] using the electric arc, stated that ultraviolet light alone had a much more powerful bactericidal action. Parkinson[3] stated that action was most vigorous on first exposure and that the number of survivors did not bear a constant relation to the number originally present. Cernovodeanu and Henri[4] and de Voogt[5] stated that the rate of destruction was almost proportional to the square of the duration of exposure.

Materials which have been exposed to ultraviolet light, have been said to retain a bactericidal property. This paper presents the results of an investigation of that subject. Coblentz,[6] Bedford,[7] Norton,[8] and Walker and Pryer[9] reported what was called a residual germicidal action of water which had been exposed to ultraviolet light. Coblentz exposed nutrient agar plates to ultraviolet light and found that the exposed agar caused appreciable mortality of *Escherichia coli.* Bedford stated that radiation of media before inoculation inhibited the growth of microorganisms to a varying degree. Norton observed a lower count in radiated water but was never able to kill all of the bacteria, even when the count was as low as 100 per cc. He could observe no action after an hour. Walker and Pryer believed that a residual action was imparted to water by the light.

In our experiments 10 organisms were used: *Serratia marcescens, Escherichia coli, Escherichia acidi-lactici, Bacillus alcaligines, Micrococcus epidermis, Bacillus megatherium, Micrococcus roseus, Bacillus subtilis, Micrococcus varians,* and *Bacillus vulgatus.* To test the

[1] Downs, A., and Blunt, T. P., *Proc. Roy. Soc.*, 1877, **26**, 488.

[2] Ward, H. M., *Phil. Trans. Roy. Soc. London*, 1894, **185**, 961.

[3] Parkinson, N. F., *Ann. Rept. Prov. Bd. of Health*, Ontario, Can., 1914, **33**, 156.

[4] Cernovodeanu and Henri, *Comp. Rend.*, 1910, **149**, 365.

[5] Voogt, J. G. de, *Z. Hyg.*, **81**, 62.

[6] Coblentz, W. W., and Fulton, H. R., *U. S. Bur. of Stand. Sci. Papers*, 1924, **19**, 495.

[7] Bedford, T. H. B., *Brit. J. Exp. Path.*, **8**, 437.

[8] Norton, *A. J. P. H.*, 1928, 476.

[9] Walker and Pryer, *A. J. P. H.*, 1921, **2**, 703.

direct action of the light, agar plates were streaked with each of 10 organisms and exposed immediately to ultraviolet light for 6 minutes, half of the plate being covered with cardboard. For testing the residual action agar plates were poured, half of each plate radiated, and both halves of the plate streaked with the test organisms. Three sets of plates were made; one set was exposed for 6 minutes, another for 30 minutes, and the third for 60 minutes. All plates were incubated for 24 hours at 37°C., after inoculation.

Exposure of agar for 6 minutes to ultraviolet light did not impart residual germicidal property for the microorganisms used. Exposure for 30 minutes caused the agar to possess slight germicidal action for *Escherichia coli, Micrococcus epidermis,* and *Micrococcus roseus,* and a marked action for *Bacillus subtilis, Bacillus megatherium,* and *Bacillus alcaligines.* The other microorganisms seemed to be unaffected. Exposure of the agar for 60 minutes produced the same results, except that *Escherichia coli* was somewhat more inhibited by exposure for 60 minutes than for 30 minutes.

For studying the question with water, attempts were made to use a large and small number of bacteria. Fifty cc. of distilled and tap water were exposed in an 8-inch petri dish. An exposure of 6 minutes was found to be just as efficient as one of 30 minutes or longer. The water was at a distance of 25 cm. and varied in temperature between 36° and 42°C. The exposed water was transferred to sterile flasks by sterile pipettes. Nine cc. of the water were inoculated with 2 dilutions of the cultures and plates prepared at regular intervals. Control plates were made with water sterilized by passage through a Seitz filter. Some of the microorganisms died as quickly in the control water, which had not been treated with ultraviolet light, as in that which had been treated. This was the case with *Serratia marcescens, Escherichia coli, Escherichia acidi-lactici, Micrococcus varians,* and *Bacillus vulgaris. Bacillus subtilis* died rapidly in both waters, but showed a slightly higher death rate in the radiated water. The other species used showed a distinctly higher mortality in the radiated water.

Why the 5 species died so rapidly in the plain water was not studied experimentally. It may have been due to a toxic factor in the water or to some physical factor such as plasmoptysis.

An attempt was then made to study this question with yeasts. Several investigators have shown that ultraviolet light is toxic for yeasts. (Tanner and Feuer[10]; Lindner[11]; Nadson and Philippov.[12])

[10] Tanner and Feuer, *J. Ind. Eng. Chem.,* 1920, **12**, 740.

[11] Lindner, *Woch. Brau.,* 1922, **39**, 166.

[12] Nadson and Philippov, *Comp. Rend. Soc. Biol.,* 1928, **366**.

The last mentioned investigators showed that ultraviolet light when administered under the proper conditions stimulated growth and caused increased budding.

For studying the residual bactericidal action of radiated water for yeasts, 3 species were used: *Willia anomala, Saccharomyces ellipsiodeus* and *Pichia polymorpha.* They were handled in the same manner as described above for the bacteria. No residual germicidal property could be established for these 3 species. They died just as rapidly in the control water as in that which had been radiated.

Conclusions. If water and other materials exposed to ultraviolet light retain a bactericidal property, it varies greatly for different organisms. There is some doubt in the authors' minds as to whether the mortality of certain bacteria in radiated water is really due to a toxic factor contributed by the ultraviolet light.

5276

Is Ferratin Precipitinogenic?

LUDVIG HEKTOEN AND WILLIAM H. WELKER.

From the McCormick Institute for Infectious Diseases and the Laboratory of Physiological Chemistry, College of Medicine of the University of Illinois.

In 1893 Schmiedeberg[1] announced that it was possible to extract a protein from the liver by means of boiling water. The extract was filtered and the filtrate treated with tartaric acid for precipitation of the protein. Schmiedeberg concluded that this was a high iron containing protein but not a nucleoprotein. He named it ferratin. Subsequent investigators,[2] however, came to the conclusion that the protein extracted in this fashion was really a nucleoprotein, or derived from nucleoprotein.

Since by repeated extraction and precipitation it seemed probable that ferratin could be completely separated from the accompanying blood and lymph proteins, it seemed advisable to test its antigenic properties.

Our first experiment was unsuccessful and new ferratin was prepared with particular care to avoid its exposure to any undue hy-

[1] Schmiedeberg, *Arch. f. exp. Path. u. Pharm.*, 1893, **33**, 101.

[2] Beccari, cited from *Malys Jafresber*, 1902, **32**, 494. Wohlgemuth, *Z. f. Physiol. Chem.*, 1902, **37**, 474, and 1904, **42**, 519. Scaffidi, *Z. f. Physiol. Chem.*, 1907, **54**, 448. Salkowski, *Z. f. Physiol. Chem.*, 1908, **58**, 282.

droxyl ion concentration. In dissolving the ferratin it was suspended in water and 0.5% sodium carbonate solution was added slowly with constant stirring in such quantities that the solution showed only the faintest reaction to litmus. No attempt was made to dissolve completely all the suspended material and the undissolved portion was removed by filtration.

Numerous experiments were made to determine whether ferratin is precipitinogenic. As the rule 2, 4, 6, 8, 10 or 12 cc. of the 1% solution of ferratin were introduced intravenously in rabbits at intervals of 3 or 4 days and on the fourth day after the last injection the serum was tested for precipitin by the contact or layer method. In all such tests progressive dilutions of the ferratin solution were tested in order to make sure that the presence of precipitin did not escape observation through the occurrence of a negative prozone. In most of the experiments no evidence of precipitin for ferratin was obtained. The serum of 2 rabbits which had received hog ferratin gave apparently specific reactions with that ferratin in dilutions of 1 to 800-1600. The serum of a rabbit treated with sheep ferratin gave precipitin reactions with beef, hog and sheep ferratins in dilutions of 1 to 3200-6400, but not with any of the corresponding serums. In another experiment the antiferratin serum reacted with sheep ferratin only at a dilution of 1 to 3200. All the dilutions of ferratin were made with physiological salt solution. Of rabbits treated with beef ferratin, the serum of one reacted with beef ferratin 1 to 1600 but not with beef serum, albumin, euglobulin or pseudoglobulin; in a second rabbit the serum reacted also with beef serum albumin; in the case of a third rabbit, the serum reacted with beef and sheep ferratins at 1 to 1600-3200 and not with beef serum, beef blood proteins or sheep serum.

From these results it may be concluded that the preparations of ferratin used in the experiments were practically free from blood proteins and possessed at most only comparatively slight precipitinogenic power, the resulting precipitins in some cases reacting also with other ferratins than the one used as antigen. The question whether ferratin is a species-specific antigen has not been settled definitely.

5277

Influence of An Exclusive Meat Diet on the Human Intestinal Flora.

JOHN C. TORREY.

From the Department of Public Health and Preventive Medicine, Cornell University Medical College, New York.

In connection with a comprehensive clinical and laboratory investigation of the effect of an exclusive meat diet on man, conducted under the auspices of the Russell Sage Institute of Pathology, an unusual opportunity was presented for a study of the reaction of the intestinal flora to such a diet. Two subjects (A and S) of these experiments continued on this diet for over a year and the third (D) for 10 days. The prescribed dietary included the choice of many cooked meats, both fat and lean portions. The protein daily intake ranged from 85 to 180 gm. and the carbohydrate (in the meat) from 5 to 10 gm. For a general statement in regard to dietary and clinical conditions reference is made to the report of C. W. Lieb[1] and for diet analyses to that of W. S. McClellan and E. F. DuBois.[2]

The fecal specimens of all 3 subjects, while on the meat diet, were of much the same character, consisting of finely divided, compact material, greyish-green, with a mild acid aromatic odor and very seldom at all offensive. The reactions ranged from neutral to moderate acidity (pH 7.0 to 6.0) and in no instance were frankly alkaline.

The direct microscopic counts of the total numbers and principal bacterial types, as differentiated by morphology and reaction to the gram stain, yielded similar findings for all. The change from the mixed to a whole meat diet caused an abrupt drop in the total bacteria, amounting approximately to 50%. In subject A this decrease was particularly marked and during the thirteenth month reached 76%. It was apparently due principally to the suppression of the lactic acid producing types such as *Lactobacillus acidophilus,* enterococci and streptococci and to a less extent, *B. coli.* Free spores and spore-bearing bacteria remained at approximately the same level but spirochaetes, numerous in one subject, disappeared soon after the change to meat.

The cultural procedures were such as to permit a comprehensive quantitative and qualitative analysis of the changes induced in the

[1] Lieb, C. W., *J. Am. Med. Assn.*, 1929, **93**, 20.

[2] McClellan, W. S., and DuBois, E. F., *J. Biol. Chem.*, 1930, **87**, 651.

colonic flora, including both the aerobic and anaerobic bacteria. The cultural findings in their main features were much alike for all 3 subjects during the meat periods, showing a general reduction in the number of viable bacteria and a suppression of the obligate fermentative organisms but on the other hand there occurred no overgrowth of putrefactive anerobes, no increase in the proteolytic propensities of the flora as a whole, except for the transitory predominance of *B. proteus* in subject A, and no introduction of unusual types of intestinal organisms.

Aside from the suppression of *Lactobacillus acidophilus*, enterococci and a group of organisms growing only under conditions of reduced oxygen tension, the *B. coli* count decreased for all subjects reaching with A after 3 months on meat one-fourth that of the mixed diet period. The hemolytic *B. coli* types were at first greatly favored by the meat diet, constituting during the first weeks from 40 to 100% of the total *B. coli* colony count. They later disappeared. There was some increase in indol-producing properties but no increase in virulence. There was a distinct tendency for the sucrose-splitting types of *B. coli* to become and remain predominant during the meat periods.

As far as spore-bearing anerobes were concerned the meat diet in all 3 individuals favored particularly the *B. welchii*. With A, during the first 3 months the estimated count was from 10X to 10 million X that of the pre-meat period and for S during the first 5 months the increase amounted to 100 X to 10,000 X. After a year on the meat diet, however, the *B. welchii* counts were no higher and, at times, lower than for the pre-meat period. Proteolytic anerobes were not added to the flora by the meat diet and the native ones were apparently not stimulated to greater activity. In fact the native types belonged in the weakly proteolytic group of which *B. bifermentans* was the representative in S. This organism tended to disappear after several months on the meat diet. Tests of the proteolytic activity of the whole flora on a cooked minced meat substrate did not show any increase over that of these individuals while on a mixed diet. Aside from the transitory overgrowth of *B. proteus* with A there was, in fact, no evidence that with individuals with normal digestion a long continued exclusive meat dietary favored the development of putrefactive conditions in the colon. It should not be assumed, however, that the same findings would hold for individuals with defective gastric or intestinal digestion and assimilation.

5278

Inhibitory Effect of Jaundice on the Growth of the Tubercle Bacillus.*

MAST WOLFSON AND EDWIN W. SCHULTZ.

From the Department of Bacteriology and Experimental Pathology, Stanford University, and Monterey Hospital, Monterey, California.

During the past year we have carried on studies to determine whether whole bile, or any one of its components, exercise growth inhibiting effects on the human, bovine, and avian types of *B. tuberculosis* either *in vitro* or *in vivo*. To determine the former possibility tubercle bacilli were grown on various media containing ox-bile, as well as on glycerine agar containing various percentages of cholesterol and cholic acid, respectively.

The *in vivo* experiments were carried out in guinea pigs and rabbits. While ox-bile, suspensions of cholesterol, as well as of cholic acid were administered intraperitoneally and intravenously, procedures which suggested themselves in part by the observations of earlier investigations.[1, 2, 3, 4, 5, 6] Furthermore, experimental jaundice was induced in a series of rabbits by ligation of the common bile duct. Some of these animals were then given intravenous injections of virulent tubercle bacilli; others were similarly injected with bacilli grown on cholesterol and cholic acid media. Unjaundiced controls were given corresponding inoculations.

As has already been pointed out by Calmette[1, 2] tubercle bacilli grow well in the presence of ox-bile, the tendency being to lose their acid fastness if kept transplanted on such media for longer periods of time. Tubercle bacilli grown on glycerine agar mixed with varying quantities of cholesterol, however, do present interesting cultural responses. Good growth is observed on ½ to 1½% cholesterol glycerine agar, there being well defined retardation when 2 to 5% is reached. It is interesting to note that this retardation in growth persisted for about 8 weeks, after which the organisms pro-

* Supported in part by the Wyborg Research Foundation, Monterey Hospital, Monterey, California.

1 Calmette, A., and Guérin, C., *Compt. rend. Acad. d. sc.*, 1908, **147**, 1456.

2 Calmette, A., Tubercle bacillus infection and tuberculosis in man and animals, Eng. tr. Soper, W. B., and Smith, G. H., Baltimore, 1923. Williams and Wilkins, 689 pp.

3 Chamberlain, E. N., *J. Physiol.*, 1928, **66**, 249.

4 Grasselle, A., *Arch. di path. clin. Med.*, 1929, **8**, 1.

5 Hinze, V., *Z. f. Tuberkheilk.*, 1928, **52**, 199.

6 Shope, R. E., *J. Exp. Med.*, 1928, **48**, 321.

ceeded to grow in a relatively normal manner. The reason for this preliminary inhibition with subsequent normal growth is not as yet clear to us. What seemed to be mutation forms similar to some described by Sweany[7] presented themselves in part of the cultures thus treated. That they were not contaminants seems to have been fully ruled out. These experiments are to be repeated shortly with the sodium salt of cholic acid added in varying percentages to glycerine agar.

In the guinea pigs, attempts to produce jaundice by tying off the common bile duct met with failure, since these animals invariably died of shock by the fifth day. This we found later had already been pointed out by Hewlett.[8] The injection of whole bile intravenously and intraperitoneally likewise proved futile. Rabbits, however, survived bile duct ligations nicely. Rabbits so jaundiced, and given intravenous injections of bovine tubercle bacilli, survived longer and at autopsy showed less in the way of lesions than the controls. Jaundiced rabbits inoculated with tubercle bacilli grown on varying percentages of cholesterol glycerine agar showed about as extensive lesions as the controls. Forty-five rabbits and 16 guinea pigs were used in these studies.

The studies are being continued, both with reference to the influence of jaundice on experimental *B. tuberculosis* infections and the influence of bile and bile derivatives on cultures in the test tube. A special point will be made to also study the influence of artificially induced hyper-cholesteria in rabbits on the course of experimental tubercle bacillus infections in these animals.

5279

Relation of Calcium and Phosphorus of Diet to Toxicity of Viosterol.*

DAVID H. SHELLING.

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University.

The discrepancies apparent in the literature as to the toxicity of viosterol may be attributed to (1) differences in the potency of the

[7] Sweany, H. C., *J. Am. Med. Assn.*, 1926, **87**, 1206.

[8] Hewlett, A. W., Pathological Physiology of Internal Diseases. D. Appleton and Company, 1923, 336 pp.

* The work reported in this and the succeeding papers was made possible by a grant from the Mead Johnson Company, Evansville, Indiana.

viosterol, (2) the character of the diet in regard to its content of calcium and phosphorus, (3) the age of the animals at the beginning of the experiment and (4) the duration of the experiments. Thus, many published results are based on the use of viosterol of unknown potency and varying methods of assay; and the diets employed range between an undescribed so-called "normal diet", through the Steenbock and McCollum rachitogenic diets, to diets consisting of "table-left-overs".

In the present study an attempt was made to ascertain the relation of calcium and phosphorus of the diet to the toxicity of viosterol.

The rats used were of our own breeding stock and were put on the experimental diets at the time of weaning. The viosterol was incorporated in the diet; the dosage was calculated in cod liver oil equivalents, *i. e.*, the amount of viosterol which produced a line test in 5 days, when added to the Steenbock rachitogenic diet, equaled ¼% of the diet in terms of cod liver oil potency.

(a) Steenbock-Bills stock diet. Ca = 0.515, P = 0.450 gm. %.

The toxicity, duration of life and well-being of animals on this diet are directly proportional to the dose of viosterol. 40,000 to 80,000 times overdosage is very rapidly fatal, sometimes with little evidence of metastatic calcifications, especially the 80,000 times overdosage. On 10,000 and 20,000 times overdosage animals do well at first, then they begin to decline in health and finally die, showing calcification of most organs, especially of the vascular system. Those on 2,000 and 4,000 times overdosage were killed at 10 months. They had reproduced many times and had shown no calcification.

(b) High calcium, low phosphorus diet. (Steenbock rachitogenic diet.) Ca = 1.240, P = 0.243 gm. %.

Since the usual length of life of an animal on such a rachitic diet is about 2 to 3 months, and since they do not grow on this diet, the deaths in this group cannot be entirely attributed to smaller doses of viosterol. On the contrary, some of the animals' lives were prolonged for long periods by adding viosterol to the diet (3 animals lived on the Steenbock diet plus 2000 times overdosage for 10 months without evidence of hypercalcification of organs). In high dosages, however, the addition of viosterol is quite fatal, but very few of the animals developed hypercalcification, although they had hypercalcemia.

(c) Optimal† phosphorus, low calcium diet. (Ca = 0.012, P = 0.475 gm. %.)

† The minimal and optimal amounts for growth and maintenance.

Animals on this diet do not grow well, but seem to survive longer than animals getting a very high calcium and the same amount of phosphorus and viosterol in the diet. With very large doses of viosterol another factor is brought into play—that of removing calcium from the bones and shifting to the blood. These animals develop emaciation, extreme osteoporosis‡ and high serum calcium concentrations. Animals on intermediate doses of viosterol show slight calcic changes in aorta and kidneys if left on the diet for a long time. Animals on this diet plus 400 times overdosage were killed at 340 days and showed very slight changes in aorta while the kidneys were normal.

(d) High calcium, optimal phosphorus. Ca = 1.212, P = 0.475 gm. %. (The amount of phosphorus is twice as great as in the Steenbock rachitogenic diet.)

Animals on this diet receiving viosterol die much sooner than on the low calcium diets. Those on very high viosterol die too soon to show calcification of organs, but those on the smaller doses which survive longer show marked calcic changes in 150 days.

(e) High phosphorus, low calcium diet. Ca = 0.012, P = 1.780 gm. %.

Animals on this diet do not grow well. On the larger doses of viosterol they do not survive very long and show moderate or mild calcification. Rats on 20, 400, and 4,000 overdosage were killed at 220 days and showed only a few specks of calcification in the corti-co-medually zones of the kidneys. These renal lesions were also seen in those animals receiving no viosterol, and are apparently due to the constant excretion of phosphate by way of the urine.

(f) High phosphorus, minimal† calcium diet. Ca = 0.412, P = 1.780 mg. %.

This group of animals was very susceptible to viosterol. They died very quickly and showed most profound calcification. The kidneys were literally made up of calcium. Even 400 times overdosage was quite fatal. With the exception of 0 and 20 times overdosage, all the animals of this series died or had to be killed because of extreme illness at 3 months or sooner. Those on 0 or 20 times were killed at 216 days and showed the kidney lesions as in the previous group.

These experiments indicate the following facts:/The composition of the diets with respect to their calcium and phosphorus content is

‡ Some of the bones in this group show extreme porosity of the upper part of the tibia just below the cartilaginous zone and appear as a pseudo-rachitic metaphysis roentgenologically.

a determining factor in the toxicity and hypercalcifying property of viosterol. In the presence of a calcifying agent, increasing the amount of phosphorus in the diet renders the organism more susceptible to hypercalcification. With large doses of viosterol animals on certain diets may die without showing any signs of hypercalcification, although hypercalcemia may be present; while on other diets they may show hypercalcification without hypercalcemia.

5280

Effect of Calcium and Phosphorus of Diet on Tetany and Serum Calcium of Parathyroidectomized Rats.

DAVID H. SHELLING.

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University.

In the study of experimental hypoparathyroidism 2 factors frequently cause contradiction and confusion: (1) the anatomical relation of the parathyroid in the animals employed; and (2) the composition of the diet, especially with respect to its calcium and phosphorus content. In experiments with the dog or the monkey the extirpation of the parathyroids without destruction of thyroid tissue is frequently impossible and the complete operation of thyroparathyroidectomy must be used. That the thyroid alone has a profound influence on calcium and phosphorus metabolism has long been appreciated and recently Aub[1] and his associates have demonstrated this fact by metabolism experiments in human beings. Hence the removal of the thyroid along with the parathyroids may cause an alteration in calcium and phosphorus metabolism different from that observed when either of them alone is removed. The rat, on the other hand, has but 2 encapsulated and easily removable parathyroids. Erdheim[2] and his followers made use of this anatomical fact and produced tetany in rats by cauterizing the parathyroids, and causing little or no destruction of the adjacent thyroid tissue. Erdheim showed that parathyroidectomy in the rat produces faulty dentition, delays calcification of a callus and impoverishes the body of lime salts.

The experiments of Erdheim were conducted prior to our more

1 Aub, J. C., Bauer, W., Heath, C., and Ropes, M., *J. Clin. Invest.*, 1929, **7**, 97.
2 Erdheim, J., *Frankfurt f. Path.*, 1911, **7**, 175.

exact knowledge of complete diets and hence no control of calcium and phosphorus intake was considered. Even in recent times the diets used in experiments on dogs and monkeys have consisted mostly of meat and biscuit and hence have been of a high phosphorus and low calcium variety. The addition of milk to such a diet or the accidental finding of calcium in infusorial earth used in metabolism experiments is looked upon by many as a faulty procedure; and milk is frequently referred to as having detoxifying properties in relieving parathyroid tetany. That the beneficial effects are due to the high calcium content of the milk must be apparent, and its mode of action has thus been explained by Salvesen.[3] It is obvious that in the normal animal the parathyroids regulate the serum calcium level, even in the face of a very small intake, but, when this regulatory mechanism is removed the small amounts of the calcium ingested are insufficient to maintain a normal serum calcium concentration, especially when the phosphorus concentration becomes elevated and part of it must be either excreted or deposited as calcium salts.

With these facts in mind, the effect of the calcium and phosphorus or the diet on the tetany and serum calcium was studied in parathyroidectomized rats. The rats were of our own breeding stock, approximately middle-aged or young adults and in perfect health. Two rats were used for each group and the experiments repeated several times at different periods, so that more than 150 rats were used. The operation consisted of the surgical removal of the encapsulated parathyroids on both sides of the thyroid while the animals were under amytal anesthesia. The diets used were "complete" except for variations in calcium and phosphorus.

The results of these experiments may be summarized as follows: (1) Animals kept on a low calcium, optimal* phosphorus diet before operation developed severe tetany within 12 to 24 hours after operation. The tetany was very violent and many died within 24 to 48 hours in *status tetanicus*. Those kept on our stock diet until the operation and then placed on the low calcium diet weathered the operation much better and tetany came on more gradually, usually appearing within 24 to 48 hours. The tetany thus produced was chronic and the animals survived for months even on the low calcium diet.

(2) If, after the establishment of tetany, 1% $CaCO_3$ was added to the low calcium diet, many of the animals gradually lost

[3] Salvesen, H. A., *J. Biol. Chem.*, 1923, **56**, 443.

* See footnote † in Paper No. 5279.

the symptoms of tetany; but severe tetany reappeared in about 5 days if the calcium was removed from the diet. The serum calcium levels in these groups varied from just above the tetanic level to as high as 12.8 mg. %.

(3) On the stock diet, tetany gradually disappeared and the serum calcium approached normal levels.

(4) On the Steenbock rachitogenic high calcium, low phosphorus diet the animals became free of tetanic symptoms very quickly and the serum calcium level varied between 11.9 and 12.9 mg. %.

(5) Animals kept on the low calcium and high phosphorus diet continued to have tetany for months and the serum calcium concentration remained at a tetanic level.

(6) On a high phosphorus and minimal* calcium diet, another factor comes into play, that of a calcium retention in the body depots. Although the serum calcium concentration may be low, the tetany frequently disappears. This will be discussed in a subsequent paper.

(7) In a few large animals cardiac puncture was performed under amytal anesthesia and blood was obtained at intervals concomitant with the change in the calcium and phosphorus of the diet. The findings corroborated the clinical symptoms and the findings observed when other animals were sacrificed for such determinations in groups. Tetany and low serum calcium concentrations were observed when the animals were on a low calcium intake; and the tetany was alleviated, and the serum calcium level rose with increase in the calcium intake.

5281

Effect of Viosterol on Serum Calcium of Parathyroidectomized Rats.

DAVID H. SHELLING.

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University.

Recently Hess[1] suggested that the effect of viosterol in raising the serum calcium level was through the parathyroids. This suggestion was based on his observations that in monkeys and dogs fed *large* doses of viosterol the serum calcium level frequently rose to 13-16

[1] Hess, A. F., Weinstock, M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 555.

mg. %. After thyroparathyroidectomy, however, *large* doses of viosterol failed to raise the calcium above the tetanic level. Greenwald[2] reports similar observations with cod liver oil and irradiated ergosterol. On the other hand, Jones,[3] Brougher,[4] Urechia and Popovicius[5] report that antirachitic agents, when given in large doses, are able to raise the serum calcium level and ameliorate the tetanic symptoms in thyroparathyroidectomized animals. The diets in these experiments are either not mentioned or are of an inconstant composition. Greenwald's diets were usually of the high phosphorus and low calcium type, and he attributes the beneficial results of vitamin D in one of his animals to an accidental mixture of calcium in the infusorial earth and that in Brougher's experiments to the administration of milk.

In the preceding paper[6] the importance of the calcium and phosphorus of the diet in relation to the serum calcium level after parathyroidectomy was stressed. In the present communication the effect of viosterol and of diet on parathyroid tetany is reported.

The results may be summarized as follows: 1. Low calcium and optimal* phosphorus diet. (a) When viosterol is added to this diet in dosages of 5% equivalent to cod liver oil† moderate elevation of the serum calcium may be observed after 20 days, and the animals continued on this diet with mild or no tetany for several months. The serum calcium levels ranged between 6.9 to 8.1 mg. %.

(b) When the dose was increased to 500%, the serum calcium levels were 7.0 and 8.3 mg. % in 24 days; 8.8 in 30 days and 10.6, 12.2 and 13.0 in 65, 73 and 90 days.

(c) With 20,000% viosterol the serum calcium was 11.0 mg. % in 8 days and the tetanic symptoms disappeared.

2. Minimal* calcium and phosphorus diet. (a) With 5% of viosterol the serum calcium level attained 11.0 mg. %. (b) With 500% viosterol, the serum calcium concentration was 7.6 and 8.4 mg. % and, within 3 months, it ranged between 8.6 and 12.6 mg. %.

A large animal of this group was bled by cardiac puncture on the second day after the operation and maintained on a low calcium diet. The serum calcium was 9.3 mg. % and tetany had not yet ap-

[2] Greenwald, I., and Gross, J., *J. Biol. Chem.*, 1929, **82**, 505.

[3] Jones, J. H., *J. Biol. Chem.*, 1926, **70**, 647.

[4] Brougher, J. C., *Am. J. Physiol.*, 1928, **84**, 583.

[5] Urechia, C. I., and Popovicius, G., *Compt. rend. Soc. de biol.*, 1928, **98**, 405.

[6] Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 301.

* See footnote † in Paper No. 5279.

† Dosage throughout these experiments is given as percent of the diet and so calculated as if cod liver oil of equal antirachitic potency were used.

peared. On the 8th day the serum calcium fell to 5.1 mg. % and tetany was severe. 1.0 mg. $CaCO_3$ and 500% viosterol were then added to the diet and the animal killed after 82 days. The animal was free of tetany and the serum calcium at the time was 9.1 mg. %.

3. Animals on stock diet in which 100% viosterol was incorporated lost the tetanic symptoms quite readily and after 60 days the serum calcium concentration was 10.0 mg. %.

4. On a high calcium, optimal* phosphorus diet plus 1000% viosterol, the serum calcium ranged between 8.5 and 12.7 mg. %.

5. With a low calcium, high phosphorus diet, and 1000% viosterol the serum calcium concentration was 9.2 mg. % in 60 days and tetany disappeared.

6. When 1% $CaCO_3$ was added to the above high phosphorus diet, the serum calcium was 10.0 mg. % in the same length of time.

Thus, in the absence of the calcium regulatory apparatus, the parathyroid gland, the shift of the calcium from the blood is so affected that ordinary dosages of viosterol, which are efficacious in normal animals, only partly or slightly affect the serum calcium levels of the organism deprived of its parathyroids. With larger doses and the removal of the additional strain, that of a deficiency in calcium in the diet, viosterol is capable of restoring the normal serum calcium level even after parathyroidectomy, provided treatment is continued for some time. It is worthy of note that the period of administration of viosterol in Greenwald's experiment lasted only 4 to 5 days—a period too short to expect an effect in an animal handicapped by thyroparathyroidectomy and by a calcium deficiency in the diet.

Recently Hess[7] also reports that he was able to raise the serum calcium level in thyroparathyroidectomized dogs and monkeys, if the previously reported large dose of viosterol was increased many times.

These experiments would seem to indicate the independent activity of viosterol. While in a normal animal it may act through and its activity be regulated by the parathyroid glands, in the absence of these glands, it may raise the serum calcium level to normal in the presence or absence of a calcium deficiency in the diet, provided large doses are used.

[7] Hess, A. F., Weinstock, M., and Rivkin, H., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 298.

5282

Effect of Diet and Viosterol on Calcium Deposition in the Callus of Parathyroidectomized Rats.

DAVID H. SHELLING.

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University.

Canal[1] showed that calcium fails to be deposited in the callus following fracture in parathyroidectomized rats, and Morel[2] noticed a similar defect in cats. Erdheim[3] concluded that the deficient calcification in the callus throughout the skeleton of parathyroidectomized rats resembles that of rickets and osteomalacia. The above experiments were conducted when exact knowledge of dietary requirements were wanting and hence, there is a possibility that the diet may have contributed to the failure of calcification in the callus in the presence of a parathyroid deficiency. The following experiments were conducted to ascertain the effect of alterations in the calcium and phosphorus diet and of viosterol on callus calcification.

The procedure and diets have been briefly described.[4] The radii and ulnae of rats were fractured while under amytal anaesthesia. The rats were placed on a low calcium diet until tetany developed and then they were divided into groups according to the diet they were to receive. X-rays of the fractured bones were taken weekly until the rats were killed.

The results may be summarized as follows:

1. (a) *Low calcium diet.* In spite of the low calcium diet the calcification of the callus progressed moderately within 30 days as evidenced by X-ray. The serum calcium in this group remained low and the animals continued to have tetany.

(b) When 1% $CaCO_3$ was added to the diet, the calcification in the callus seemed somewhat denser.

(c) *Stock diet.* Very good calcification of the callus was observed within 20 days.

(d) *Steenbock rachitogenic diet.* In spite of its being a rickets-producing diet, the calcium deposition in the callus was marked.

(e) *High phosphorus and low calcium diet.* The deposition of calcium salts was very poor, even after a month.

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[1] Canal, A., *Gazz. d. osp.*, 1909, **30**, 977.

[2] Morel, L., *Compt. rend. Soc. de Biol.*, 1911, **63**, 749.

[3] Erdheim, J., *Frankfurt Z. f. Path.*, **7**, 175.

[4] Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 301, 303.

2. (a) Low calcium diet with 5% viosterol. Callus formation fair, somewhat better than with low calcium diet alone.

(b) When 500% viosterol was added to the above diet the calcifying effect in the callus was very apparent.

(c) When 1% $CaCO_3$ and 500% viosterol were added to a high phosphorus and low calcium diet, the calcium deposition in the callus was definitely increased, but the degree of deposition did not approach that of a normal callus or a callus in the experiments in which less phosphorus and the same amounts of calcium and viosterol were used.

Unoperated animals kept on a stock diet showed nearly complete healing of fractures in 18 days.

The results seem to indicate again the importance of diet in parathyroidectomy experiments. They prove clearly that in the presence of a parathyroid deficiency calcification does not occur regularly, if calcium is also lacking in the diet. They also show that the property of viosterol in elevating the serum calcium level after parathyroidectomy is evident in its ability to promote callus calcification. In comparison with the normal animal, however, callus calcification is somewhat delayed.

5283

Role of the Parathyroids in Calcification, and Susceptibility of Parathyroidectomized Rats to Viosterol.

DAVID H. SHELLING.

From the Harriet Lane Home of the Johns Hopkins Hospital and the Department of Pediatrics, Johns Hopkins University.

The long continued administration of parathyroid extract to animals results in an increased excretion of calcium and phosphorus from the body.[1, 2, 3] Similarly, the presence of hyperfunctioning parathyroid glands results in a negative calcium and phosphorus balance and bone decalcification termed clinically, *osteitis fibrosa cystica*. Several observers noticed marked improvement when one or more of these glands was removed. Hence, in hypofunction a marked retention of calcium and phosphorus should result. Greenwald,[4] contrary to previous assumptions, demonstrated retention of

[1] Greenwald, I., Gross, J., *J. Biol. Chem.*, 1926, **68**, 325.

[2] Bodansky, A., Blair, J. E., Jaffe, H. L., *J. Biol. Chem.*, 1930, **88**, 629.

[3] Albright, F., Bauer, W., Ropes, M., and Aub, J., *J. Clin. Invest.*, 1929, **7**, 139.

[4] Greenwald, I., Gross, J. J., *J. Biol. Chem.*, 1925, **66**, 185.

these elements after thyroparathyroidectomy in dogs. The validity of Greenwald's conclusions may be questioned because the thyroid extirpation may have contributed to this retention. However, in metabolism experiments on rats, in which the parathyroids alone were removed, retention of calcium and phosphorus was observed, provided the diet contained a certain amount of these elements,[5] thus substantiating, in part, Greenwald's hypothesis. However, no attempts have as yet been made to determine the depots of retention of calcium and phosphorus. That their deposition is not always in the osseus tissue is evidenced by the observations of Erdheim,[6] Iselin,[7] and Toyofuku.[8] Leopold and Von Reuss[9] found that the combined soft tissues of parathyroidectomized rats contained more calcium than those of control rats.

In the course of study on the effect of calcium, phosphorus and viosterol intake on parathyroidectomized rats it was noted that they were much more susceptible to viosterol hypercalcification than normal animals. A dose of viosterol, which ordinarily would not produce calcification in a normal rat in a given length of time, may cause calcification in the organs of the parathyroidectomized animals, provided certain amounts of calcium and phosphorus be present in the diet. The effect was absent when the calcium intake was very low, and more pronounced when the intake of phosphorus was high and that of calcium at optimal levels.

It was also observed that parathyroidectomized animals receiving no viosterol, but which were on diets containing calcium with normal or high phosphorus concentrations, developed calcification of their vascular system, especially the aorta and the vessels of the kidney. One animal developed extensive calcification of both lungs.

It was shown[10] that normal animals are more susceptible to viosterol overdosage when their diet contained minimal calcium and high phosphorus concentrations. The increased intake of phosphorus apparently caused a transitory or permanent increase of this element in the body and, in the presence of calcium and calcifying agent, such as vitamin D, these ions in excess of the solubility product constants are deposited in the tissue as the insoluble salt. A similar mechanism may operate in the parathyroidectomized organism. It was also shown[4, 5] that the immediate effects of depri-

[5] Shelling, D. H., to be published.

[6] Erdheim, J., *Frankfurt Z. f. Path.*, 1911, **7**, 175.

[7] Iselin, H., *Deutsche Z. f. Chir.*, 1908, **93**, 494.

[8] Toyofuku, T., *Frankfurt Z. f. Path.*, 1911, **7**, 249.

[9] Leopold, J. S., and Von Reuss, A., *Wien. klin. Wchnschr.*, 1908, **21**, 1243.

[10] Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 298.

vation of the parathyroids is the retention of phosphorus and decrease or an absence of this element in the urine. The decreased excretion of calcium, which follows, may be the result of the parathyroid extirpation primarily or it may be due to the increased phosphorus retention and the retained calcium is thus deposited in the tissues as the insoluble salt.

The relation of hypoparathyroidism to abnormal calcification such as in Paget's disease, juvenile sclerosis, renal sclerosis, otosclerosis, and general arteriosclerosis is worthy of consideration. The association of a low serum calcium and low calcium excretion in otosclerosis has been frequently observed by otologists; and the occurrence of cataracts in parathyroid tetany in human beings and experimental animals has been noted by many observers. The association of extreme calcification of the vascular system and renal disease has been noted clinically and pathologically and it is possible that the parathyroid plays a rôle in this syndrome.

The rôle of the parathyroids and high phosphorus diets, such as meat, in general arteriosclerosis of the middle aged can only be speculated upon at present.

5284

Note on Individual Differences in Human Blood.

K. LANDSTEINER AND PHILIP LEVINE.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

1. Previously an agglutinable property of human blood designated as P was described, which could be demonstrated by the use of immune agglutinins from rabbits,[1] and it was mentioned[2] that reactions of a more or less similar specificity occur with a certain type of irregular human isoagglutinins. Inasmuch as these 2 reagents are not always easily available it seems worth mentioning that similar results can readily be obtained with various absorbed normal animal sera, *e. g.*, those of horses, pigs and rabbits. In horse serum the agglutinins were found rather frequently.

2. An anti-dysentery immune serum from a goat was shown by Eisler[3] to contain agglutinins for human blood which can be ab-

[1] Landsteiner, K., and Levine, Philip, *J. Exp. Med.*, 1928, **47**, 757.

[2] Landsteiner, K., and Levine, Philip, *J. Immunol.*, 1930, **18**, 91.

[3] Eisler, M., *Z. f. Immunitätsf.*, 1930, **67**, 38.

sorbed by dysentery bacilli (Shiga). Using this serum, kindly supplied to us by Eisler, we were able to confirm fully these interesting observations. Furthermore we found that after absorbing the serum with cells of the sub-group A^1 the supernatant fluid agglutinated most cells of sub-group A^2, O, and probably B, distinctly more strongly than those of sub-group A^1, in a similar way as certain exceptional human sera.[4] Whether this is a singular occurrence or can be reproduced with other dysentery sera is being investigated.

5285

The Oligodynamic Haemolytic and Haemagglutinative Properties of Some of the Heavy Metals.

SANFORD B. HOOKER.

From the Department of Immunology, Evans Memorial, Boston.

An accidental observation that $HgCl_2$ in high dilution was powerfully lytic for erythrocytes suspended in an isotonic medium led to the collection of data concerning the effect of many of the commoner salts in concentrations ranging from 0.01M to 0.0000003M. Progressively doubled dilutions in 0.85% NaCl solution were mixed with equal quantities of washed human cells in 2% suspension, placed, with a fragility test, in a water bath at 37°C. for 1 hour and read finally after some 20 hours' refrigeration at 8°C. The effect of the active salts was decidedly more in evidence at the 20 hour than at the 1 hour reading. The positive results recorded do not pretend to quantitative accuracy because of the considerable variations sometimes observed in repeated tests with cells of slightly different fragility.

Some salts were tested also with sheep and with rabbit cells; the latter were rather more sensitive to lysis and were involved in a peculiar phenomenon that was not observed with the other 2 types of erythrocytes. In the weakest partially effective lytic dilutions of the mercury salts the unhemolyzed cells were gathered in a tough film.

The hemolytic effect of the mercurials was surprisingly little diminished by the presence of even an excessive quantity of serum in the isotonic diluting medium.

[4] Landsteiner, K., and Levine, Philip, *J. Immunol.*, 1929, **17**, 8, 11.

Figure 1 shows the nature, extent, and zonal qualities of the positive effects observed. In the higher concentrations many salts produced discoloration which is not here recorded.

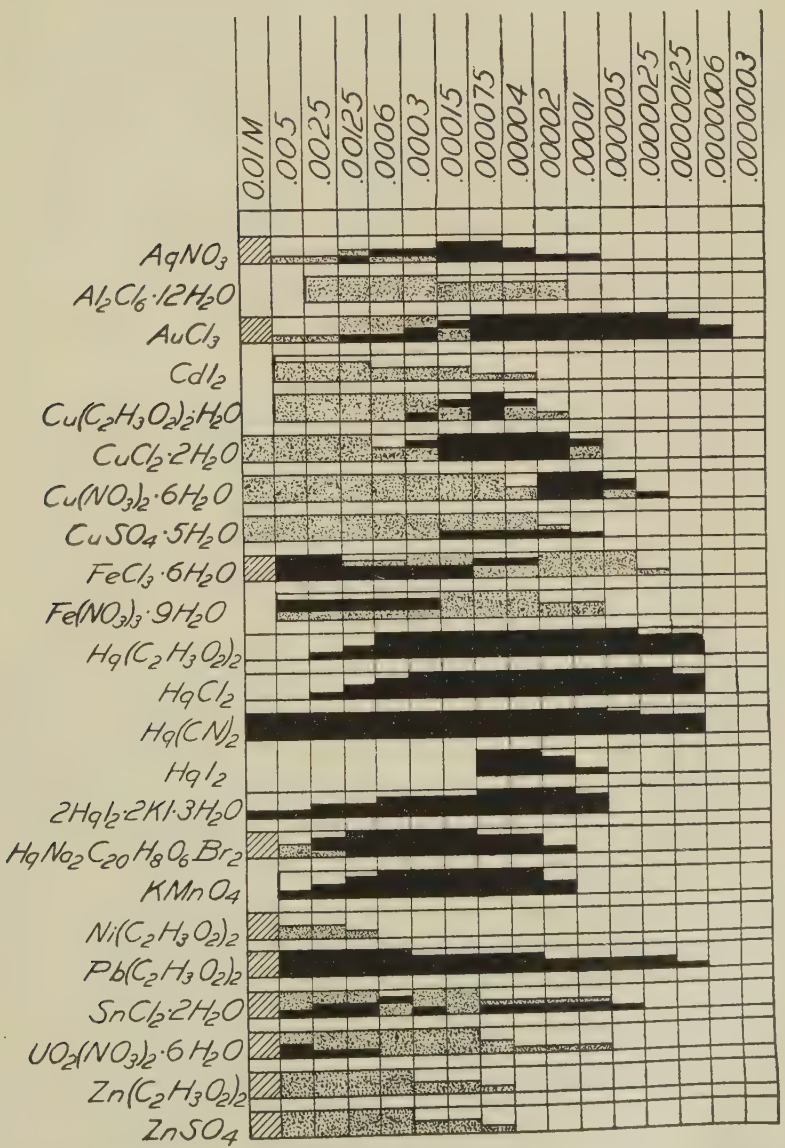

FIG. 1.

Black areas represent lysis; stippled, agglutination; blank, no visible change; barred, not tested. The varying heights of the black and stippled areas indicate degrees of reaction—from partial to complete.

Negative results were obtained with colloidal gold, $BaCl_2.2H_2O$, $CaCl_2$, $Co(NO_3)_2.6H_2O$, $FeSO_4(NH_4)_2SO_4.6H_2O$, K_3AsO_4, $KAsO_2$, KCl, KCN, $K_2C_2O_4.H_2O$, K_2CrO_4, $K_4Fe(CN)_6.3H_2O$, $KHCO_3$, K_2HPO_4, KI, KIO_3, KNO_3, K_3PO_4, K_2SO_4, $K_2S_2O_8$, Li_2CO_3, Li_2SO_4, $MgCl_2.6H_2O$, $MgSO_4$, $MgSO_4.7H_2O$, $NaBO_2$, $Na_2B_4O_7.10H_2O$, NaF, $Na_2Fe(CN)_5NO.2H_2O$, $Na_2HPO_4.12H_2O$, $NaNO_2$, $Na_2S_2O_3.5H_2O$, $Na_2WO_4.2H_2O$, NH_4Br, $NH_4C_2H_3O_2$, NH_4Cl, $(NH_4)_2CO_3.H_2O$, $(NH_4)_2C_2O_4.H_2O$, NH_4NO_3, $(NH_4)_2SO_4$.

These observations may be of some interest possibly to students of toxicology, antisepsis, chemotherapy, permeability and allied basic problems.

5286

On the Almost Instantaneous Transport of Spermatozoa Through the Cervix and the Uterus in the Rat.

CARL G. HARTMAN AND JOSEPHINE BALL.
(Introduced by Herbert M. Evans.)

From the Department of Embryology, Carnegie Institution, and Phipps Psychiatric Clinic, Johns Hopkins Hospital.

It is almost universally believed that spermatozoa owe their progression through the female genital tract to the lashing of their vibratile tails. Elaborate experiments have been made testing the rate of progression of spermatozoa in salt solution under the microscope, their chemotaxis towards uterine, tubal and ovarian tissue, their negative rheotropism towards a ciliary stream, the time elapsing between copulation and the appearance of sperms at the fimbriated end of the tube. (Bischoff.)*

Since the uterine horns of laboratory rodents as well as other mammals are filled to turgidity with fluid at the time of oestrus, it seemed logical to try to determine whether the uterine fluid also functioned as a readily miscible matrix for the rapid transport of the semen by uterine contraction.

Preliminary experiments were made by the senior writer in the

* The senior author (C. G. H.) questioned this view[1] He stated "that spermatozoa may reach the *os uteri* through the lateral vaginal canals almost instantly by simple peristalsis of the highly motile female organ—a passive transport in the liquid of the canal."

[1] Hartman, Carl G., *Anat. Rec.*, 1924, **27**, 293.

laboratory of Dr. Herbert M. Evans, which led to no definite conclusions because of the difficulty of determining the exact instant of ejaculation. In the same laboratory, the junior writer observed and learned to interpret the behavior of the male so as almost infallibly to diagnose the copulation that resulted in ejaculation, which made the present study possible. Thanks are due Dr. Evans for the use of the facilities of his rat colony and to Dr. R. R. Squier for assistance in the present series of experiments. The results are striking and at variance with the general notion, yet clear-cut and conclusive.†

A series of 3 rats was first taken. Two, 8 and 16 minutes respectively after the effective copulation the animals were killed, the body cavity opened and Bouin's fluid poured over the uterus. This was removed after fixation was well advanced. One minute elapsed before killing and opening the body cavity. All 3 specimens had masses of spermatozoa in the distal end of each horn. The result was surprising; yet the experiment was open to the objection that the *inside* of the uterus may not have been affected in time to stop the swimming spermatozoa.

The next 4 animals were studied in the living, the animal killed either instantly or after an interval following the effective coitus; the body cavity was quickly opened and a clamp placed on the uterine horns near the distal end, leaving a small portion at the extreme tubular end for testing.

Experiment 1. Killed one minute after copulation. At 2 minutes the uterine horns had been clamped near apex; at 2 minutes and 18 seconds, myriads of spermatozoa taken from the cornual apices were demonstrated under the microscope.

Experiment 2. Removed from cage the fifty-fifth second after ejaculation; killed the sixtieth second; left horn clamped at 100 seconds, right clamped at 116 seconds. Sperms had reached apex in left, about half way up in right horn.

† The ejaculatory sign in the rat must first be given. Although the copulatory behavior pattern in the rat has been fully described by Stone,[2] he interpreted the behavior accompanying ejaculation as a "minor irregularity". The rat copulates several times (5 to 20) before actually ejaculating, withdrawing suddenly and then usually licking the penis. When ejaculation occurs, however, the last of the four or five pelvic thrusts is deeper than usual and the male lies on the back of the female, withdrawing slowly only at the end of several seconds. The deep final thrust, then, followed by a period of inertia on the part of the male, lasting several seconds, constitutes the sign of ejaculation. It is practically infallible; nevertheless, to make even more certain that there be no mistake, the females were frequently examined to rule out any premature ejaculation.

[2] Stone, Calvin P., *J. Comp. Psych.*, 1922, **2**, 95.

Experiment 3. Killed 30 seconds after copulation. Left horn clamped 24 seconds later, the right 9 seconds after that. No sperms were found distal to the clamps, but plenty of them in the lower half of each uterine horn.

Experiment 4. Killed as quickly as possible after mating. Right horn clamped 54 seconds, left 63 seconds after coitus. Plug with active sperms in vagina, none in cervix or uterus.

From the last experiment one might conclude that something has to happen in the genital tract of the female within a few seconds of the effective coitus to effect the entrance of the semen into the cervix.‡ However, the experiments as a whole show conclusively that once the sperms enter the rat uterus they are rapidly carried, by contraction of the uterine wall itself, quite passively throughout the uterus to its distal end and the very portal of the fallopian tube. The transport of sperms, at least in the uterus of the rat, is a matter not of hours or of minutes, but of seconds. It is easy to observe contraction waves in the uterus at this period. Parker,[3] however, described the rabbit uterus as sluggish and concluded from his experiments that 2 hours was the time required for the journey covered in a half-minute in the rat.

5287

Implantation of Juvenile Testicular Tissue into the Hypertrophied Right Gonad of Ovariotomized Fowl.*

L. V. DOMM. (Introduced by Frank R. Lillie.)

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

In our early ovariotomy experiments[1] sex-inversion was incomplete owing to the fact that we failed to find spermatogenesis in the hypertrophied testis-like right gonad, though the individuals were otherwise equipped to function as males. In a subsequent series of experiments[2] in which the operations were performed at an earlier

‡ A similar observation was made by Parker[3] in the rabbit.

[3] Parker, G. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 826.

* This investigation was supported in part by a grant from the committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

[1] Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 28; *J. Exp. Zool.*, 1927, **48**, 31.

[2] Domm, L. V., *W. Roux' Archiv. f. Entw. mechan.*, 1929, **119**, 171.

age, the majority under 4 weeks, some cases of spermatogenesis have been found. In these cases the spermatic tubules are almost invariably isolated and confined to very limited areas of the gonad so that such poulardes are probably rarely, if ever, capable of functioning as males. In order to determine whether the poularde could function as such special experiments were devised.

Sixteen poulardes showing masculine characteristics, particularly head furnishings, were selected. These had been operated at a relatively late age, hence were not likely to reveal spermatogenesis. Laparotomies were performed and into the right testis-like gonad of each juvenile testicular tissue was placed Two short incisions were made on the exposed surface of the gonad approximately 8 mm. apart. By means of a curved probe a tunnel was made between these and the tissue introduced. This technique was essential since it relieved the pressure which, with a single incision and tunnel, invariably expelled the tissue introduced. The birds were kept under observation from 10 to 14 months when they were killed and examined.

Mating tests were conducted which proved negative, no fertility having been found over a period of approximately 5 months. None of the birds were actually known to tread, though their behavior otherwise was characteristically masculine. External characterization remained typically poularde, no perceptible modifications appearing in plumage, head furnishings, or spurs. Cases showing successful implantations appeared no different from those unsuccessful. Except in one case, which showed pronounced hypertrophy due to growth of engrafted tissue, the right gonads did not differ macroscopically from controls. Vasa deferentia all revealed growth, some having become definitely coiled, though none were better developed than those found in the normal poularde. The oviducts likewise were characteristic of the normal poularde. Gonadal smears were made in each instance on post-mortem, 3 of which proved positive. These showed numerous motile spermatozoa.

Serial cross sections were made of the entire gonad in each of the 16 cases. Histological examination revealed spermatogenesis in 5 cases. The spermatic tissue in these cases revealed the general character of testis grafts being principally composed of the following types of tubules. (1) Tubules containing cells in all stages of maturation without mature spermatozoa. (2) Tubules similar to the first but containing spermatozoa. (3) Tubules showing various degrees of desquamation of germinal epithelium. (4) Sterile tubules similar to those which characterize the testis-like gonad of the normal poularde and also found in parts of the host gonad. The com-

parable tissue of the host gonad was composed of the usual cords and tubules.

The distribution of the tubules of the graft within the host gonad varies. In some the tubules of the graft are rather definitely confined in certain parts of the gonad, while in others they may be quite uniformly scattered, while another condition was that in which the tubules were arranged in small scattered aggregations. In places the engrafted tubules were rather conspicuously partitioned from those of the host. The extent to which the host gonad had been penetrated by the implanted tissues is variable. In 1 case practically the entire gonad had been invaded while in the rest the invasion has not been so extensive, though in none does less than one-third of the series reveal some tubules of the graft. The abundance of these in any particular cross section varies from a single tubule to those in which practically the entire tissue is composed of seminiferous tubules.

That the poularde has the potentialities of developing a complete male genital system from gonad to cloaca cannot be questioned, for occasionally such a system may be demonstrated. However, in the present series a preliminary study has failed to demonstrate a connection between the tubules of the implanted gonad and the efferent system of the host gonad. Evidence for spermatic infection of the tubules of the host gonad is difficult to determine.

5288

Artificial Insemination with Motile Sperm from Ovariotomized Fowl.*

L. V. DOMM. (Introduced by Frank R. Lillie.)

From the Whitman Laboratory of Experimental Zoology, University of Chicago.

Our recent studies[1] have demonstrated the occurrence of spermatogenesis in the ovariotomized Leghorn fowl and explained the conditions under which it arises. In these cases ovariotomy was performed at a relatively early age before the primordial germ cells in the right rudimentary gonad were known to disappear. In a

* This investigation was supported in part by a grant from the committee for Research in Problems of Sex of the National Research Council; grant administered by Prof. Frank R. Lillie.

[1] Domm, L. V., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 338; *W. Roux' Archiv. f. Entw. mechan.*, 1929, Bd. 119, p. 171; *Anat. Rec.*, 1929, **42**, 15.

small percentage of the birds operated under these conditions spermatogenesis was later found to occur.

These early cases of spermatogenesis were found on histological examination of the serially sectioned gonad. This study revealed that only exceedingly rarely did most of the gonad undergo spermatogenesis, but that almost invariably only a small part of the tubules of the gonad revealed this condition, while the rest of the gonad was composed of scattered sterile tubules varying in number. In a few cases spermatogenesis was apparently not yet complete in that no differentiated spermatozoa were found. A continuous passageway between the spermatic tubules and the associated vas deferens could not be positively demonstrated, though such connections with some of the tubules may occasionally be found in sterile right gonads. In all cases belonging to the series, the anterior part of the vas deferens from its area of attachment to the gonad to 5-6 mm. posteriorly was sectioned. This part of the duct was always fixed intact with the gonad, care being taken not to destroy the union between them. In no case has sperm been found in this part of the duct.

The question whether the spermatozoa observed in such cases of sex-inversion are actually motile and capable of fertilization is exceedingly interesting and one that obviously could not be answered from a study of the histologically prepared material. Furthermore, since the poularde is not known to tread except in very rare cases, having found but one case in our entire work, special methods were necessary to determine this point. Hence the surviving poulardes of the series were subjected to special post-mortem examination. Deep transverse incisions were made at close intervals throughout the entire gonad and several smears from each examined microscopically. Smears of the contents of the vas deferens near the gonad were also examined.

Twenty-eight poulardes were examined. In each of these the contents of the vas deferens were devoid of spermatozoa. Smears from each of the gonads excepting one were likewise devoid of spermatozoa. This case, Bird No. 1084, yielded motile spermatozoa approximately 6 mm. from the posterior end; the entire gonad measuring 1.8 cm. in length and 1.1 cm. greatest width. These were not numerous but their vibrations were unmistakable and some could be clearly seen in the cellular debris of the smear. The poularde had been ovariotomized when 8 days old and was killed 3 years and 17 days later. It had become typically masculine after the manner of ovariotomized fowl. It, however, had not yet re-

vealed any indication of plumage reversion, having retained the luxuriant male plumage throughout.

The part of the gonad containing spermatozoa was carefully removed from the rest and placed in a sterile dish with about 6 cc. of Locke's solution and thoroughly mashed. Equal parts of the sperm suspension were then introduced by means of an ordinary medicine dropper into the cloaca and distal end of the oviduct of 3 virgin brown Leghorn laying hens. All eggs laid were collected for 18 days and incubated. No fertility was found. A similar technique, previously developed, in which spermatozoa were obtained from the vasa deferentia of sexually active cocks yielded fertile eggs.

It is highly probable that the sperm suspension was not sufficiently dense to bring about fertility under the conditions involved. It is also probable that the spermatozoa of the fowl may undergo an ageing or ripening process in some part of the reproductive system other than the seminiferous tubules which is essential for fertilization. If this were true then spermatozoa taken directly from the seminiferous tubules would be decidedly inferior to those, for instance, found crowded in the vasa deferentia of a sexually active cock. According to Young[2] such a condition actually prevails in mammals, though it has not been demonstrated in the bird.

5289

A Quantitative Study of Ovulation in the Rabbit.*

J. M. WOLFE. (Introduced by R. S. Cunningham.)

From the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee.

Evidence presented by Smith and Engle[1] points to the fact that there is a correlation between the amount of gonadal stimulating hormone of the anterior lobe of the hypophysis and the stages of the oestrous cycle. These investigators found that only a slight response was secured from glands taken from donors during the oestrous period, while a marked reaction was secured when glands were taken from animals which were in dioestrous.

[2] Young, Wm. C., *Anat. Rec.*, 1929, **44**, 252.

* This investigation has been aided by a grant from the Committee on Research in Problems of Sex of the National Research Council.

[1] Smith, P. E., and Engle, E. T., *Anat. Rec.*, 1929, **42**, 38.

We have tested the capacity of the anterior lobe of the hypophysis of the sow, taken at different periods of the oestrous cycle, to induce ovulation in the rabbit. Using the work of Corner[2] as a basis, it is possible to select sows at the different periods of the oestrous cycle. The head and the entire reproductive tract of these sows were brought to the laboratory from the killing floor within 30 minutes after the death of the animals. The hypophysis was removed and the anterior lobe dissected out and carefully weighed. It was ground up very thoroughly with sand in physiological saline, and centrifuged for 30 minutes. The supernatant fluid was drawn off and diluted to a point where 1 cc. of the fluid represented 5 mg. of anterior lobe tissue. These manipulations were carried out under sterile conditions. The follicles and corpora of the donor's ovaries were carefully measured and the ovaries were fixed in Bouin's fluid for histological study. Blocks from the uterus and the vagina were also fixed.

The saline suspension obtained from the anterior lobe tissue was injected intravenously in sexually mature female rabbits in various amounts. On the basis of 144 experiments, the following conclusions have been reached:

1. Injections of anterior lobe tissue in amounts as small as 1 mg. induced ovulation in rabbits when the ovaries of the donor contained inactive corpora and follicles measuring from 6-8 mm. in diameter. 2. Injection of 20 mg. were required when the ovaries of the donors contained inactive corpora and follicles measuring up to 10 mm. 3. It was necessary to inject 40 mg. of anterior lobe tissue when the ovaries of the donors contained large active corpora and small follicles in the resting condition.

On the basis of these experiments, it seems possible to conclude that the anterior lobe of the hypophysis of the sow undergoes cyclic physiological changes.

Ovulation has been induced in pregnant rabbits by the intravenous injection of anterior lobe suspension. These experiments have not as yet been quantitated.

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[2] Corner, G. W., *Contributions to Embryol.*, 1921, **13**, 117.

5290

Eye Hypersensitivity Elicited by Monilia Psilosis Polysaccharide.

E. MOTT AND H. D. KESTEN. (Introduced by J. W. Jobling.)

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

In studies on sympathetic ophthalmia, Woods[1] sensitized one eye of dogs by injecting uveal pigment into the vitreous humor and observed an inflammatory reaction in both eyes on subsequent intraperitoneal injection of the same antigen. By injecting horse serum about the iris, Riehm[2] succeeded in sensitizing the rabbit eye so that subsequent intravenous injection of the antigen produced a bilateral uveitis. In their work on local organ hypersensitiveness Seegal and Seegal[3] sensitized the anterior chamber of the rabbit eye with various protein antigens, and later obtained local reactivation by intravenous administration of the homologous antigen.

Inasmuch as the substances heretofore used to sensitize and reactivate the eye have been protein in nature, a similar experiment is reported, using as the reactivator a polysaccharide fraction[4] prepared from the yeast-like fungus *Monilia psilosis,* Ashford. The organism itself was used as sensitizer, since the uncombined bacterial polysaccharides have been found to be non-antigenic. Suspensions of washed heat-killed monilia organisms, both intact and ground, were injected into the anterior chamber of one eye of each of 12 rabbits* after removal of a somewhat greater volume of aqueous humor. Control animals were given sterile saline or a 5% saline solution of the monilia polysaccharide. At various intervals (2 weeks to 8½ months) after the original injection, and after the transitory inflammatory reaction had subsided, monilia polysaccharide, usually 50 mg. in 5% solution, was injected into an ear vein. When a positive reaction occurred, it reached a maximum in 5 to 7 hours after the injection, and was characterized by marked

[1] Woods, A. C., *Arch. Ophth.*, 1918, **47**, 161; *J. Am. Med. Assn.*, 1921, **77**, 1317.

[2] Riehm, W. D., *Med. Woch.*, 1929, **55**, 907.

[3] Seegal, D., and Seegal, B. C., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 390.

[4] Cook, D. H., Kesten, H. D., and Jobling, J. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 562; Kesten, H. D., Cook, D. H., Mott, E., and Jobling, J. W., *J. Exp. Med.*, 1930, **52**, 813.

* Dr. A. L. Morgan of the Department of Ophthalmology, Presbyterian Hospital, New York City, very kindly instructed us in the eye technique and assisted with some of the clinical observations.

hyperemia of the circumcorneal conjunctival vessels and often of the vessels of the iris and nictitating membrane.

The usual incubation period of 2 weeks was not sufficiently long for reactivation of the eye to be successful. This was probably due to the continued presence of antigen in the eye. Antigen was often visible grossly for as long as 10 days. One positive eye reaction was noted at the end of 2 months. Tested 4 or 4½ months after the original eye injection, 5 of the 12 rabbits exhibited a positive reaction in the sensitized eye. The opposite eye was invariably negative and served as a control. Of these 5 animals one was positive as well at the end of 2 months and of 4 months. Control animals were negative, as were also those rabbits sensitized with monilia but given monilia bodies intravenously instead of polysaccharide. One of the latter exhibited a definite eye reaction 2 weeks later when given polysaccharide. Five animals tested at 8½ months were negative, although 3 of these had yielded good reactions at 4 months. No difference was noted between rabbits sensitized with whole and with ground monilia.

Inasmuch as the polysaccharide fraction used in the work contained, as previously reported,[4] a small amount of nitrogen (averaging 0.6% in the samples used), it cannot be stated unqualifiedly that the monilia-sensitized rabbit eye can be reactivated by subsequent intravenous injection of the homologous protein-free polysaccharide. The negative character of protein tests would indicate, however, that very little, if any, of the nitrogen is present as protein. Furthermore, nitrogen-free pneumococcus carbohydrate has been found capable, by Avery and Tillett,[5] of producing anaphylactic shock in passively sensitized guinea pigs. We are inclined, therefore, to interpret the eye reaction to the monilia substance as another manifestation of the ability of a bacterial hapten to elicit an immune reaction in the properly sensitized medium, in this case the actively sensitized eye.

[5] Avery, O. T., and Tillett, W. S., *J. Exp. Med.*, 1929, **49**, 251.

5291

I. Effects of Injected Extracts of Fresh Pineal Glands of the Cow on Growth of Immature White Mice.*

S. J. WEINBERG AND A. F. DOYLE. (Introduced by J. J. Abel.)

From the Department of Pharmacology, Johns Hopkins University.

The rôle of the pineal gland in growth both before and after puberty is still a controversial subject, and it was with the hope of throwing some light on the question that these experiments were performed.

The mice used were bred in this laboratory from a selected standard stock, this procedure being essential to insure uniform results. Injections were begun after weaning at 21 days of age and continued for periods of 28 days. Conditions of diet, temperature, and cages were kept uniform for each group of 20 mice used. As a further check each litter was kept in a separate cage. Injections were made 3 times a week subcutaneously, each injection of 0.15 cc. containing 2 mg. of protein material and being equivalent to 0.03 gm. of gland. Weight measurements were made before each injection. In the preparation of the extract, sterile technique was used throughout except for sterilization of the extract itself, the method of preparation being to grind the material with twice its weight of clean sand, extract with distilled water and centrifuge. Control injections of sterile physiological saline were made.

At the end of 24 days of injection, during which time no retardation or acceleration of growth beyond that of the group of 20 control mice injected with physiological saline was observed, the mice, with no evidence of infection, showed a characteristic syndrome, became cachetic, and died within a day or two. To check again on the method of preparation and injection 2 additional series of 20 mice each were injected respectively with neutralized acid and alkaline extracts of the fresh gland of the cow. The protein content was made the same as before. In the case of the neutralized acid extract injections no cachexia or death was had at the end of a 28-day period. On the other hand, the toxic principle seems to go over into the alkaline extract inasmuch as this on being neutralized and injected leads to cachexia. Growth on injection of either neutralized acid or alkaline extracts is again neither accelerated nor retarded. One constant feature of this condition of cachexia is the

* An investigation carried out under a grant from the Carnegie Corporation of New York.

assumption of a gross reddish brown appearance by the adrenal glands. Microscopic examination failed to bring out anything of significance, when sections of the reddish brown glands were compared with those of normal, cream colored adrenal glands. Fat stains of frozen sections were not made. The controls and mice injected with neutralized acid extract of equal protein content do not give this appearance of the adrenals.

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II. Effects of Injected Extracts of Fresh Pineal Glands of the Young Calf on Growth of Immature White Mice. Effects on Sexual Apparatus.*

S. J. WEINBERG AND R. V. FLETCHER. (Introduced by J. J. Abel.)

From the Department of Pharmacology, Johns Hopkins University.

Interest in this phase of the problem was stimulated by the clinical correlations of sexual precocity with tumors of the pineal gland. The experimental conditions were maintained exactly as for the preceding experiments. Some variation was necessary in that the young calves' pineal glands required were unobtainable in Baltimore, it being necessary to have them shipped on ice once weekly from Armour and Company in Chicago, to whom we are indebted for this valuable material. The glands were in a perfect state of preservation for preparation of the extract 48 hours after being removed from the animals. The extract was kept frozen between injections to preserve it for the remainder of the week. There was observed neither stimulatory nor retarding effect on the growth of immature white mice. A toxic principle was found in this case also on injection of the saline extract and the 20 animals after a preliminary period of apparent perfect health presented a condition of cachexia to all appearances identical with that mentioned in the preceding paper. Sufficient material was not available to determine the effect produced by alkaline or acid extracts. The weights of the testicles of the males taken in proportion to body weight after a period of injection of 24 days were found to be neither increased nor diminished beyond the weights of testicles of control mice injected with physiological saline over a like period.

* An investigation carried out under a grant from the Carnegie Corporation of New York.

5293

Renal Damage Following the Administration of Inorganic Phosphate.

EATON MAC KAY AND JEAN OLIVER.

From the Scripps Metabolic Clinic, La Jolla, California, and the Department of Pathology, Long Island College of Medicine, New York.

In an earlier communication[1] it was reported that the addition of acid or basic sodium phosphate was followed by a remarkable increase in the size and weight of the kidneys. Further experiments have demonstrated that these changes take place with the addition of an adequate quantity of inorganic phosphate in many forms to the diet of young albino rats. These include the sodium and potassium salts, either primary, secondary, tertiary, or neutral mixtures and phosphoric acid itself. The phosphate concentration used in most of the experiments was essentially the same as that which was used in the earlier experiments[1] with a 4 calorie per gram diet, although a quarter of this concentration was found to produce abnormal kidney changes. / An examination of the histological structure of these kidneys, 250 in all, has made us aware of interesting pathological changes.

The lesions noted in the kidneys of all the animals irrespective of variation in the nature of the salt fed, was essentially similar and consisted of epithelial destruction with later reparative processes. Cloudy swelling and necrosis of the cells of the convoluted tubules followed by regeneration were noted in all of the various experiments, including those of from 3 days' duration to the longest period used (44 days). Associated with these processes there occurred calcification of the necrotic debris which accumulated as a result of them so that calcified tubular casts filled the tubule lumen. In those animals that had been fed the phosphate diets for considerable periods of time, proliferative processes were also noted in the interstitial connective tissue about those tubules which had suffered most severely.

All these changes were localized to a very definite portion of the kidney, namely the outer zone of the medulla where the terminal portions of the proximal convoluted tubule dip into the substance of the medulla. / The cortical tissue remained normal, except in the longest experiments where secondary changes of tubular collapse

[1] MacKay, L. L., MacKay, E. M., and Addis, T., PROC. SOC. EXP. BIOL. AND MED., 1926, **24**, 130.

were noted. Further experiments are being designed to study the exact relations of these lesions to the feeding of phosphate salts.

5294

The Innervation of the Human Suprarenal Glands.

LOUIS K. ALPERT. (Introduced by Raymond Hussey.)

From the Department of Pathology, Yale University School of Medicine.

In the course of a study of the intraglandular innervation of the suprarenal glands in man, the following observations were made. By the Spielmeyer technique, it was found that the nerves approaching the glands were myelinated, and many continued so through the cortex, but no myelinated nerves were observed in the medullary portion of the glands. The finer nerve fibers, which came into intimate relation with the cells of the glands, were found to be unmyelinated. By the original Nissl technique, numerous ganglion cells were found, both on the surface of the glands, and within the medulla, especially in the region of the hilum; none, however, were observed in any of the cortical layers. The Bielschowsky technique was employed in the study of the finer distribution of the nerve fibers. The cells of the *zona glomerulosa* were seen to be supplied by short fibrils directly from the nerves in the capsule. Longer fibers passed down between the columns of the fascicular zone, anastomosing abundantly, and forming networks which enclosed the cells and sent tiny branches to end within them. These fibers supplied the cells of the *zona reticularis* in a similar manner. To the medulla, the large nerve bundles described as myelinated were observed to pass directly, with no evidence of branching within the cortex. The ganglion cells described under the Nissl technique were seen to possess intracellular fibrils, while the medullary cells themselves were enclosed in basket-like networks of fibrils, from which tiny fibrillae were seen to enter the cells.

5295

Fluctuations of the Concentration of "Blood-sugar" in Vitro.*

ISRAEL S. KLEINER.

From the Department of Physiological Chemistry, New York Homeopathic Medical College and Flower Hospital, and the Biological Laboratory, Cold Spring Harbor, L. I.

In previous reports we have discussed the behavior of the blood-sugar under various conditions. It was first shown[1] that when blood from diabetic dogs was dialyzed against Ringer's solution the sugar dialyzed out at an irregular rate as compared with normal blood containing added glucose. (Hirudin, or novirudin, was used as the anticoagulant in all experiments.) In some instances, and especially when the determinations were made at short intervals, the curves were most irregular, sometimes apparently indicating a formation of sugar under the influence of dialysis. We therefore subjected diabetic blood to short periods of dialysis, after which the blood was transferred to a glass vessel and blood-samples were taken every 15 or 20 minutes. These blood-sugar curves also showed irregularities[2]; sometimes figures were obtained which exceeded the original values before dialysis. We have since studied this phenomenon further and wish to report the observation that this irregularity following dialysis almost invariably occurs in diabetic blood (dog or human) particularly if the initial sugar value is quite high. With concentrations of 270 mg. per 100 cc. or less, the fluctuations are not as marked, or may even be absent.

As a matter of routine we then conducted control experiments in which the blood was not subjected to dialysis or any other procedure, expecting a slow, regular, downward trend, due to glycolysis. We were surprised to find similar fluctuations in both dog and human diabetic blood, again noting that often the original blood-sugar value was exceeded and also that the greatest fluctuations occurred at the higher levels. Even normal blood to which glucose was added yielded similar irregular curves, but if no glucose was added there occurred only the regular glycolysis.†

The curves are not uniform in character; the peaks and troughs

* Aided by a grant from the Littauer Foundation.

1 *J. Biol. Chem.*, 1918, **34**, 471.

2 *Am. J. Physiol.*, 1929, **90**, 410.

† Thirty-one experiments were performed on blood from 12 diabetic patients; 25 on blood from 3 diabetic dogs; 18 on blood from 11 non-diabetic patients; and 18 on blood from 9 normal dogs.

do not fall at corresponding points. The fluctuations range from 10 to 40 mg. per 100 cc. (or even more) in 15 or 20 minute periods and are not due to the activity of microorganisms. Further studies on the source, identity, and behavior of this reducing substance are in progress.

5296

Value of Hexuronic Acid in the Treatment of Graves' Disease with Suprarenal Cortex.

DAVID MARINE, EMIL J. BAUMANN AND BRUCE WEBSTER.*

From the Laboratory Division, Montefiore Hospital, New York.

In 1921[1] and in 1924[2] we reported 20 cases of Graves' disease treated with a glycerol extract of fresh ox suprarenal cortex. This treatment produced very striking improvement in 12 to 15 days in the following particulars, namely (1) a gain in body weight, (2) a gain in muscle strength, (3) disappearance of diarrhea, (4) control of excessive menstrual flow and (5) a marked decrease in the metabolic rate, approaching normal after 2 to 4 months.

During the past 6 years we have treated approximately 50 additional cases by this method with the same general results.

In 1928 Szent-Györgyi[3] isolated a hexuronic acid from suprarenal cortex which is easily destroyed by exposure to air. In the preparation of our suprarenal cortex emulsion any hexuronic acid present would probably be destroyed. We, therefore, thought that possibly more effective results might be obtained if our treatment with suprarenal cortex were supplemented by concentrates of freshly prepared hexuronic acid. The following are the results obtained to date:

Case 1. Female, married, age 32, No. 18012. Patient had acute rheumatic fever in 1926 and diphtheria in July, 1929. Following the diphtheria, the patient noted weakness, palpitation, swelling of the ankles and sweating. Thyroid enlargement was noted in August, 1929, for which she was treated with Lugol's solution for 3 weeks. On admission the patient had all the symptoms of a severe Graves' disease in addition to a rheumatic cardiac disability.

* Fellow National Research Council.

1 Marine, D., and Shapiro, S., *Endocrinol.*, 1921, **5**, 699.

2 Shapiro, S., *Endocrinol.*, 1924, **8**, 666.

3 Szent-Györgyi, A., *Biochem. J.*, 1928, **22**, 1387.

During a preliminary rest period of 2 months there occurred the expected decrease of metabolism from +62% to +41%. Between January 4 and January 22, 300 mg. of hexuronic acid was administered daily with no further decrease of metabolism. Between January 22 and May 29, 12 to 24 cc. of suprarenal cortex emulsion was given daily by mouth. The basal metabolic rate decreased from +41 to +15%. Between May 29 and October 30, no further medication. The B.M.R. then was 0, the pulse rate had dropped from the admission rate of 112 to 68 and the weight had increased from 48 to 57 kg.

Case 2. Another patient treated in a similar way gave similar results.

Case 3. Female, Admission diagnosis of Graves' disease. Patient has palpitation, muscle weakness, loss of weight and thyroid enlargement. She thinks these symptoms began to appear about one year ago.

During a control period (2 weeks) of rest in bed, patient showed no change. Between April 3 and June 12, 12 cc. of the cortex emulsion was given daily by mouth, and the B.M.R. dropped from +77 to +42%. Between June 10 and July 2, 400 mg. of a hexuronic acid concentrate was given daily by mouth. The B.M.R was not measured during this period, but the pulse rate remained unchanged. Between July 2 and August 24, 24 cc. of the suprarenal cortex emulsion was given daily. The B.M.R. sharply dropped to +3. The pulse was 72, while the weight had increased from 40 to 53 kg.

Two additional patients were given about 200 mg. hexuronic acid plus 24 to 36 cc. suprarenal cortex emulsion daily. They improved at the same rate as patients treated with suprarenal cortex emulsion alone.

In a sixth patient, the effect of hexuronic acid alone was noted. This patient, male, age 43, No. 18567, was admitted with a diagnosis of Graves' disease, May 19, 1930. He states he has always been nervous and temperamental but that active symptoms of Graves' disease date from the stock market break in October, 1929.

A larger dose of hexuronic acid concentrate (400 mg. twice daily) was given by mouth. Between May 19 and June 14 the B.M.R. showed no change, remaining at +33%. Pulse rate dropped slightly from 96 to 81 while weight increased 2.5 kg. After a quiet summer his B.M.R. was +25. Pulse rate was 84 while his weight had increased 11 kg.

This evidence so far shows that hexuronic acid is not the agent

of the suprarenal cortex which is so strikingly beneficial in Graves' disease. It is possible, however, that parenteral administration of hexuronic acid may be effective.

This evidence strongly supports the conclusion reported in our previous papers that (1) a symptom-complex essentially identical with Graves' disease, in its physiological and pathological aspects, can be produced by sublethal injury of the suprarenal cortex of rabbits and cats;[4, 5] (2) a glycerol extract of suprarenal cortex consistently causes strikingly beneficial effects in Graves' disease; (3) this suprarenal cortex extract as shown in 1926[6] lowers the metabolism of rabbits when thyroids are intact and (4) when hexuronic acid from suprarenal cortex is substituted for the glycerol extract of suprarenal cortex, no beneficial effects occur. We are convinced that the primary etiological factor in Graves' disease is the deficiency of the internal secretion of the suprarenal cortex.*

5297

On the Nature of the Dye Penetrating Nitella from Cresyl Blue.

MARIAN IRWIN.

From the Laboratories of the Rockefeller Institute for Medical Research.

(1) Measurements of the sap of *Nitella B* with the glass electrode made with actively growing cells collected in early summer confirmed those formerly obtained[1, 2] and showed that there were no seasonal changes affecting the results. Living cells were placed in cresyl blue solution at pH 9 until 0.07% dye had penetrated the vacuole, causing an increase of about 0.4 pH in the sap. This corresponded to the increase when 0.07% dye base was dissolved in the sap *in vitro,* but not to the behavior of the dye salt which did not raise the pH value. These results show that the dye does not penetrate as a salt (undissociated DCl or $D^+ + Cl^-$).

[4] Marine, D., and Baumann, Emil J., *Am. J. Physiol.*, 1922, **59**, 353.

[5] Scott, W. J. M., *J. Exp. Med.*, 1922, **36**, 199.

[6] Marine, D., Baumann, Emil J., and Cipra, A., *Am. J. Physiol.*, 1925, **72**, 248.

* There is abundant evidence that the gonads contain a similar substance.

[1] Through the kindness of Dr. D. A. MacInnes and Dr. M. Dole the applicability of the glass electrode method to the studies of single cells was tested. *J. Gen. Physiol.*, 1928-29, **12**, 805.

[2] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 132; *J. Gen. Physiol.*, 1930-31, **14**, 1.

They also show that in case *D* ions penetrated by an exchange of cations, they must have exchanged with H ions to account for the increase in the pH value of the sap. In this case more H ions must diffuse than K ions. But this is very improbable, owing to the fact that the concentration of H ions is several thousand times lower than that of the K ions in the sap while there is no indication that the mobility of the H ions in the protoplasm is very different from that of the K ions. It must therefore be concluded that the dye penetrates as a base. Whether it does so in an undissociated form or as an ion pair must be left undecided.

(2) If the dye penetrates as *D* ions we should expect their mobility in the protoplasm to be very high since the dye penetrates rapidly even from a very dilute solution. This was tested by measurements of potential difference on living cells, employing 0.01 M $CaCl_2$ and cresyl blue at pH 8 and pH 9 made up in diluted borate buffer mixtures containing no K. The pH values were checked by means of the glass electrode. Owing to uncertainty as to the absolute concentration of cresyl blue, several concentrations were used against 0.01 M $CaCl_2$ and were found to give approximately the same readings. The results show that the mobility of *D* ions is about the same as that of Ca ions which is considered by Osterhout[3] to be very low. They therefore tend to favor the idea of penetration as the undissociated base.

(3) To understand the rôle of this dye base it is necessary to consider the various components of cresyl blue solution. In the experiments on *Nitella,* roughly between pH 6 and pH 9, the external solution gives an absorption curve identical with cresyl blue and remains unaltered. But if the cresyl blue solution at a pH value above pH 8 is shaken with benzene or chloroform until a great deal of the blue dye (giving an absorption spectrum identical with cresyl blue) is absorbed, the aqueous solution appears to contain a mixture of cresyl blue and a violet dye formed irreversibly. The amount of the violet dye is so small that it does not seriously affect the determination of the partition coefficients if sufficient quantity of the blue dye is left in the aqueous solution at equilibrium (as in former experiments) nor does it affect the experiments on *Nitella.* Other irreversibly formed substances at higher pH values appear too far outside the range to play any part in the experiments on *Nitella.* The same may be said of the oxazone[4] which is formed below pH 4 and which is not present in the sample employed. At

[3] Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715.

[4] Cohen, B., and Preisler, P. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 92.

about pH 11 the dye becomes somewhat orange but on immediate addition of acid the blue color reappears. This orange dye may be an imine base: it decomposes rapidly.

The dissociation constant[5] of the dye base which penetrates is about $10^{-5.6}$ as shown by the experiments on *Nitella* and on partition coefficients. This dye is spectrophotometrically[2] identical with cresyl blue but the name may be altered to cresyl blue X since this dye is regarded by Cohen[4] and Preisler (on the basis of oxidation-reduction potential measurements) as a decomposition product of cresyl blue while cresyl blue is regarded by them as more strongly basic. But this does not alter any of the results and conclusions heretofore obtained. On this basis a parallel may exist in the case of methylene blue[6] from which also the more weakly basic component (azure B) penetrates more rapidly than methylene blue.

5298

Effects of Posterior Pituitary Extracts on the Lactic Acid of the Blood.

H. E. HIMWICH AND J. FAZIKAS.

From the Department of Physiology, Yale University.

It has been noted that pitocin increased the metabolic rate of rats, while pituitrin and pitressin decreased the oxygen consumption.[1] In the present experiments the effects of 20 to 30 cc.* of chloretone-free solution of each of the 3 posterior pituitary extracts were observed on 3 unanesthetized and 2 amytalized dogs. Subcutaneous injections were made at half hourly intervals and blood samples drawn every 2 hours for 6 hours were analyzed for lactic acid.

The 3 unanesthetized animals that received pitressin showed a consistent increase in lactic acid so that the results of any one of these may be taken as representative. In each of the 3 experiments, by the end of the second hour lactic acid had increased at least 20 mg. per cent, and then gradually diminished until the sixth

[5] Irwin, M., *J. Gen. Physiol.*, 1925-26, **9**, 561.

[6] Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 563; *J. Gen. Physiol.*, 1928-29, **12**, 147; 1930-31, **14**, 19.

[1] Himwich, H. E., and Haynes, F. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 815.

* 1 cc. pituitrin contains 10 oxytocic and 10 pressor units. 1 cc. pitocin contains 10 oxytocic units and 1 cc. pitressin contains 10 pressor units.

EFFECT OF PITRESSIN ON LACTIC ACID OF BLOOD, mg.%.

Preinjection	2 hrs.	4 hrs.	6 hrs.
13	33	27	13

hour when the lactic acid had returned to the preinjection level. One of the 3 dogs that were given pituitrin exhibited a similar rise in lactic acid. The other 2 animals as well as the 3 which were given pitocin, had variations in the concentration of lactic acid which were within the experimental error (±2.5 mg.%). With amytal there were no significant changes in lactic acid after the administration of any of the posterior pituitary extracts. Our task is to make further studies of the character of the metabolism during the depression of the metabolic rate.

5299

Effect of Posterior Pituitary Extract on Plasma Concentration and Fat Content and on Blood Sugar.

H. E. HIMWICH, F. W. HAYNES AND M. A. SPIERS.

From the Department of Physiology, Yale University.

Raab[1] has demonstrated that injections of pituitrin diminish the concentration of plasma fat of unanesthetized animals. The present work is concerned not only with the effects of pituitrin, but also those of pitocin and pitressin on the concentration and fat content of plasma and blood sugar. Eighteen experiments were made on unanesthetized dogs and 21 were performed under amytal on dogs weighing from 6 to 24 kilos and in various nutritive conditions. The dosage of the chloretone-free extracts varied from 10 cc. to 30 cc.* Blood was drawn from the femoral artery, usually at 2 hourly intervals and analyzed for sugar by the method of Hagedorn-Jensen.[2] The fat content was determined according to the procedure of Stewart and White.[3] Table I presents the changes in fats, plus and minus indicating changes of at least 40 mg. per cent. Table II contains the effects of the posterior pituitary extracts on the total

[1] Raab, W., *Z. f. d. ges. Exp. Med.*, 1926, **46**, 179.

* 1 cc. of pituitrin contains 10 oxytocic and 10 pressor units. 1 cc. of pitocin contains 10 oxytocic units and 1 cc. of pitressin contains 10 pressor units.

[2] Hagedorn, H. E., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

[3] Stewart, C. P., and White, A. C., *Biochem. J.*, 1925, **19**, 840.

solids of the plasma. The smallest difference is 3 mg. per cc. of plasma.

TABLE I. *Changes in Plasma Fat.*

		+	0	−	Remarks
Pituitrin	Unanesthetized	1	0	5	In 2 instances a rise preceded the fall.
"	Amytalized	2	0	3	
Pitocin	Unanesthetized	4	0	3	
"	Amytalized	2	1	5	
Pitressin	Unanesthetized	3	0	2	In 1 instance a rise preceded the fall.
"	Amytalized	2	0	6	
Control	Unanesthetized	0	1	0	
"	Amytalized	0	2	0	

TABLE II.
Total Solids of Plasma.

		+	0	−
Pituitrin	Unanesthetized	0	0	7
"	Amytalized	0	0	1
Pitocin	Unanesthetized	0	0	3
"	Amytalized	2	0	2
Pitressin	Unanesthetized	0	0	4
"	Amytalized	2	0	4

TABLE III.
Changes in Blood Sugar.

		+	0	−
Pituitrin	Unanesthetized	2	0	0
"	Amytalized	2	0	1
Pitocin	Unanesthetized	5	0	0
"	Amytalized	0	0	3
Pitressin	Unanesthetized	3	0	0
"	Amytalized	0	0	3

The effects of the extracts on blood glucose are presented in Table III. Changes of glucose are at least 5 mg. per cent.

The effects of pituitrin in diminishing the concentration of the plasma and its fat content as well as in raising blood sugar are also caused by pitocin and pitressin. These actions on the blood constituents are not so marked, or are actually changed, under amytal anesthesia.

5300

Effect of Ephedrine on Blood Glucose and Lactic Acid and Plasma Fat.

H. E. HIMWICH, H. HENSTELL AND J. FAZIKAS.

From the Department of Physiology, Yale University.

The effects of subcutaneous injections of ephedrine on blood lactic acid and sugar plasma fat were studied in 10 dogs varying in weight from 4 to 18 kilos. The animals were in a post-absorptive condition and under the influence of amytal. The dosage of ephedrine sulphate (Eli Lilly and Company) in the different experiments

varied from ¼ to 1 cc. of a 3% solution, and was given every 15 minutes for from 2 to 5 hours.

The level of blood sugar was raised in every experiment. Increases in fat occurred in 7 experiments and therefore were not so consistent as those obtained with adrenalin.[1] The concentration of the lactic acid of the blood increased in only 5 experiments. The determination of the total solids of the plasma was unsatisfactory because of hemolysis, an effect of ephedrine. The results of a typical experiment are presented below.

TABLE I.
Effect of Ephedrine on Blood Glucose and Lactic Acid and Plasma Fat.

	Blood sugar	Blood lactic acid	Plasma fat	Hemo-crit	Dosage
Pre-injection	110	44	705	56	
2 hours	183	59	1120	56	½ cc. ephedrine
4 "	134	60	807	64	every ¼ hour for
6 "	125	71	696	63	4 hours.
8 "	125	48	697	64	

5301

Growth and Sexual Maturity in Daphnia magna.

B. G. ANDERSON.* (Introduced by J. H. Bodine.)

From the Biological Laboratory, Western Reserve University.

Several workers have stated that the number of pre-adult instars in Cladocera is constant within the species. Banta and Brown[1] report that *Moina macrocarpa* has 3 pre-adult instars. Agar[2] reports that *Simocephalus gibbosus* and *Daphnia carinata* have 3 and 4 respectively but adds that a small percentage of the individuals become mature in one less than the normal. Such is not the case with *Daphnia magna* which has 5 or more pre-adult instars. Calvert[3] found similar conditions for the Odonata. Sexual maturity appears to be correlated with size in *D. magna.*

[1] Himwich, H. E., and Petermann, M. L., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 814.

* The data for this paper are the results of work done at the Zoological Laboratory, State University of Iowa.

[1] Banta, A. M., and Brown, L. A., *Proc. Nat. Acad. Sci.*, 1929, **15**, 71.

[2] Agar, W. E., *J. Exp. Biol.*, 1930, **7**, 349.

[3] Calvert, P. P., *Proc. Am. Phil. Soc.*, 1929, **68**, 227

Individual females of the same clone have been reared on 2 different culture media. The animals used were isolated within 8 hours after their release from the mothers and placed in vials containing 30-36 cc. of the media. These were kept at room temperature (20°-25°C.). One of the media was fresh manure-soil medium.[4] The other was old manure-soil medium to which one drop of dilute oatmeal mush was added daily to each vial. In the former one-third of the fluid in each vial was replaced semiweekly by fresh medium.

Following isolation each individual was observed daily, cast carapaces recorded, and measurements of the body length taken of each animal. No appreciable difference was found in body length when 2 or more measurements were made on the same individual during any one instar. This is in agreement with the findings of Agar.[2]

The results show that the individual apparently must reach a certain size before it becomes adult, *i. e.*, before the first clutch of eggs appears in the brood chamber. The number of pre-adult instars is not correlated with the size of the individual during the first instar. For the one medium the number of pre-adult instars was constant at 5—the lowest number observed. For the other, the oatmeal modification, the number varied from 5 to 8. The variation in the number of pre-adult instars was probably due to the amount of food available. In the fresh manure-soil medium very few large ciliate protozoa were found, while in the other they were numerous. These probably reduce the number of smaller organisms in the culture without themselves being available as food so that the *Daphnia* went into a state of partial inanition. Similar conditions have been observed when the cultures were infested with large numbers of the annelid *Æolosoma*.

It appears, therefore, that the number of pre-adult instars is largely dependent on the food supply available. In all likelihood a minimum number of pre-adult instars exists for each species of Cladocera. An optimum food supply must be available to reach this state and any excess of this would represent that which the animal cannot assimilate.

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[4] Banta, A. M., *Science*, N. S., 1921, **53**, 557.

5302

Effect of Complete Biliary Fistula on Blood Cholesterol.

EARL GARSIDE. (Introduced by Alton Ochsner.)

From the Department of Surgery, Tulane School of Medicine, Tulane University, New Orleans, Louisiana.

The rôle of the biliary system in the metabolism of cholesterol is not well understood. Cholesterol taken in the food is largely absorbed into the blood. Some is excreted into the bile, but is to a large extent reabsorbed. The cholesterol in the faeces should theoretically be derived partly from the food, partly from the bile. Dorée and his associates concluded that the cholesterol in the faeces could be accounted for entirely by the cholesterol of the food. That cholesterol can be and is absorbed in the intestinal tract has been repeatedly demonstrated. That cholesterol can be absorbed by the gallbladder is believed by many, but has not been conclusively demonstrated.

Many experimenters[1, 2, 3, 4] have injected cholesterol and other lipoids into the gallbladder and after ligating the cystic duct have noted a degree of absorption. Boyd[5] was able to demonstrate a lowering of the blood cholesterol 9 days after cholecystectomy. Sweet,[6] however, found a rise in blood cholesterol of almost double normal after cholecystectomy, which continued to be present for about 40 days and became lower than normal only after 72 days.

It would appear logical that if bile were lost by the presence of a biliary fistula there should be a definite reduction in blood cholesterol. If cholesterol is absorbed in the gallbladder this reduction should be even more marked if a complete biliary fistula were made and the gallbladder removed also.

Attempt was made to demonstrate the changes in the blood cholesterol in dogs with a complete biliary fistula, both with and without the presence of the gallbladder. All the animals were fed a special kennel food in amounts calculated according to their body weights. Repeated daily readings of the blood cholesterol were made. The cholesterol was estimated on the whole blood by Sackett's modification of Bloor's method. The blood was withdrawn from the jugu-

[1] Illingworth, C. F. W., *Brit. J. Surg.*, 1929, **18**, 203.
[2] Aschoff, L., *Münch. med. Woch.*, 1906, **38**, 1847.
[3] Mentzer, S. H., *Am. J. Path.*, 1925, **1**, 38.
[4] Torinoumi, K., *Ziegler's Beiträge*, 1924, **72**, 456.
[5] Body, W., *Brit. J. Surg.*, 1923, **10**, 337.
[6] Sweet, J. E., *Internat. Clinics*, 1924, **1**, 187.

lar vein at the same time daily. Feedings were also at a regular hour. The food was given by gavage when not voluntarily eaten. After several normal readings were obtained, operation was done. In 4 dogs a complete biliary fistula was

TABLE I.

Dogs with Complete Biliary Fistula. Daily Determination.

Mgm. Chol. per 100 cc. blood	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Dog C 700				214.2	230	230	214	222	181	240	200	260	245	
" C 703	230	206	214.2	193.5	214	230	222	214	217	211	227	230		
" C 704	171	176	189	187	222	230	200							
" C 707	217	227	240	157	157	230								

Dogs with Complete Biliary Fistula and Cholecystectomy.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
" C 702	250	214	206.9	200	206	210	187	214	234	240	237	272	260	176
" C 705	166	166	176	174	206	214	222	214	214	211	227	214	182	
" C 706	193	116	169	166	176	160	187	166	240	238	260	285	260	
" C 708		156	163	157	250	165	176	181	176	224	208	230	224	

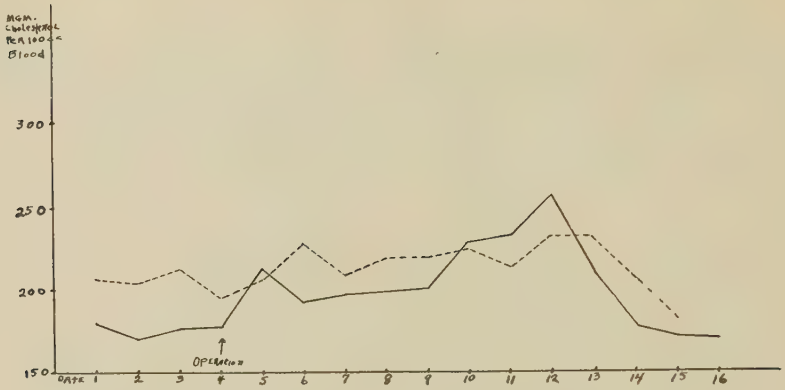

FIG. 1.
Composite curve of daily cholesterol determinations on 8 dogs.
—— complete biliary fistula and cholecystectomy.
- - - - complete biliary fistula only.

made by severing the common duct near its entrance into the duodenum, ligating the distal end, and cannulizing the proximal end with a small soft rubber catheter, the size depending on the caliber of the duct. This catheter was brought out through the abdominal wall by allowing it to go through the peritoneum at the upper angle of the incision, course in the preperitoneal fat to the lower angle of the wound, then pass through the muscle and course in the subcutaneous tissue to the upper angle of the incision and then pass through the skin. To the end of the catheter was attached a sterile rubber balloon. By this method the possibility of ascending infection of the bile ducts was minimized. In 4 other dogs an identical procedure was done and in addition the gallbladder was removed. Ether was the anesthetic used. Daily cholesterol determinations were continued (see table). The dogs lost weight rapidly and many of them died within 10 days after operation.

The increase in blood cholesterol was not as marked as that observed by other experimenters after operation on the biliary tract. There was not an appreciable difference in the blood cholesterol in dogs with complete biliary fistula with cholecystectomy and those with complete biliary fistula only.

5303

Duck Disease Caused by the Toxin of Cl. Botulinum Type C.

M. HOBMAIER. (Introduced by K. F. Meyer.)

From the Laboratory, California Fish and Game Commission, Hooper Foundation for Medical Research, San Francisco, California.

Study of the clinical manifestations and the absence of characteristic anatomical findings suggested that the so-called duck disease is the same sickness as Limber neck of chickens. The unknown different symptoms caused by Botulinus in wild and domesticated ducks may be due to the natural habits of these birds. To keep themselves from drowning the water birds stretch their heads backwards. This is of course not necessary in the case of chickens and tame ducks. Furthermore the experiments of Mr. Kalmbach refuted the theory of a salt intoxication and forcibly indicated the existence of an animated virus.

The theory of Botulinus intoxication was confirmed in a series of experiments to be detailed elsewhere. Cultures prepared from the liver and other organs of sick wild ducks contained a toxic anaerobe. 0.3 to 0.5 cc. from these cultures, administered by mouth or when injected, induced in healthy wild ducks the paralysis of legs, wings and *membrana nictitans,* respiratory distress and aphonia, strikingly similar to that in naturally diseased birds. In general the autopsy findings are negative with the exception of a dilated rectum filled with accumulated urine. Inoculation of mice revealed the typical symptoms of Botulinus. These experiments proved the identity of the duck disease and the Limber neck of chickens, chiefly produced by *Clostr. botulinum* Type C, sometimes also by Parabotulinum Type A. Botulinus Type C toxin formed by strains obtained from Limber neck in chickens, Parabotulinum toxin A, and even Type B in larger doses, are experimentally capable of producing the duck disease.

Pure cultures could be prepared without difficulty. The first culture made from a sick wild duck (Williams Lake) contained a coccus and anaerobes. The isolation of the anaerobe was obtained by heating the culture at 80°C. for 3 minutes. Morphological and biological investigations showed the presence of *Cl. botulinum* Type C. A culture from a killed experimental duck was given to Dr. K. F. Meyer and Dr. H. H. Heller. They also succeeded in isolating the *Cl. botulinum* Type C and identified it by toxin-antitoxin absorption test according to standard methods.

The same anaerobe has been found in the liver of 4 acutely fatal cases of duck disease, and in 5 birds, which had suffered from the same malady 3 weeks before they had died of an intercurrent disease. So far, in one duck recovered from duck disease 6 weeks previously the cultural demonstration failed. These observations are of great epidemiological interest in connection with the fact that generally the young birds are subject to the disease.

Minnesota Section.

University of Minnesota, November 26, 1930.

5304

Spatial Discrimination.

SHELDON K. WIRT AND ESTHER M. GREISHEIMER.

From the Department of Physiology, University of Minnesota.

Spatial discrimination on various parts of the body under approximately constant external conditions was studied in a group of 25 male medical students, and in 1 individual on 25 different occasions over a period of 4 days.

The regions tested were: (1) the tip of the tongue, (2) the volar surface of the distal phalanx of the index finger, (3) the volar surface of the second phalanx of the index finger, (4) the tip of the nose, (5) the dorsal surface of the second phalanx of the index finger, (6) the dorsum of the hand, (7) the manubrium sterni, (8) the antero-radial surface of the forearm, and (9) the back of the neck *(regio nuchae)*.

The subject was blindfolded, and the tests were made with the aesthesiometer in the usual manner. The means, standard deviations, coefficients of variation, and differences between the tongue (always the subtrahend) and the other regions, found for the 25 medical students are presented in Table I.

TABLE I.
Spatial Discrimination in 25 male students.

Region	Means (mm.)	Standard Deviations	Coefficients of variation	Differences (Tongue as subtrahend)
1	1.45±0.053	0.394±0.037	27.15	——
2	2.03±0.11	0.823±0.078	40.52	0.58±0.12
3	3.18±0.21	1.59 ±0.15	40.91	1.73±0.21
4	3.33±0.18	1.36 ±0.13	40.38	1.88±0.19
5	4.93±0.23	1.75 ±0.17	35.39	3.48±0.23
6	15.31±0.96	7.14 ±0.68	46.63	13.86±0.96
7	28.46±1.18	8.76 ±0.83	30.79	27.01±1.18
8	29.67±1.64	12.18 ±1.15	41.05	28.22±1.64
9	36.16±1.41	10.47 ±1.00	28.95	34.71±1.41

TABLE II.
*Spatial Discrimination in 1 Subject on 25 Occasions.**

Region (See text)	Means	Standard Deviations	Coefficients of variation
1	1.50±.029	0.212±.020	14.14
2	1.85±.047	0.346±.033	18.72
3	3.00±.138	1.033±.098	34.23
4	3.91±.174	1.29 ±.123	33.05
5	4.53±.177	1.31 ±.125	28.99
6	17.62±.571	4.23 ±.404	24.02
7	29.78±.863	6.40 ±.610	21.48
8	37.12±.699	5.18 ±.494	13.96
9	40.28±.649	4.81 ±.459	11.94

* The authors are much indebted to Mr. Carroll J. Bellis (Teaching Fellow in Physiology) for acting as the subject on whom 25 tests were made during 4 days. The results found for Mr. Bellis are presented in Table II.

The differences between the means for Mr. Bellis and for the 25 students were calculated, and with the exception of the forearm results, they were found to be insignificant statistically. In 6 of the regions examined (excepting 4, 5 and 7) the standard deviations found for Mr. Bellis were significantly lower than those for the group.

We wished to determine if a high sensitivity of the tip of the tongue was associated with high sensitivity in other regions of the body. For this, the correlation coefficients (ρ) between the readings for the tongue, and those for 1—the back of the neck, 2—the volar surface of last phalanx of the index finger, and 3—the tip of the nose, were calculated. The following results were found:

	Correlation Coefficient
Tip of tongue and back of neck	—.136±.132
" " " " volar surface last phalanx of index	—.006±.135
" " " " tip of nose	—.081±.134

These clearly show an absence of correlation between the sensitivity of the tongue, and of other parts of the body.

5305

Value of X-Ray Evidence of Bowel Obstruction in Various States of Intestinal Stasis.

H. A. CARLSON, H. J. DVORAK, F. W. LYNCH, C. BORMAN AND O. H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

X-ray evidence of gas in the small intestine in experimental simple mechanical obstruction and in strangulation obstruction has been reported previously.[1, 2] It was found that definite evidence of gaseous distention of the bowel proximal to a simple obstruction was present as early as 4 or 5 hours after the establishment of the obstruction and in 20 to 24 hours the intestinal distention was fairly general in the proximal bowel. The X-ray was not found to be of the same value in detecting the presence of strangulation as it was in the early recognition of simple obstruction of the intestine.

These X-ray observations have now been extended to include cases of isolated loop obstruction, mesenteric vein ligation and experimental peritonitis.

In 23 dogs, segments of intestine from 10 inches to 3 feet in length were isolated from the rest of the intestinal tract (ileum, jejunum or duodenum) and the ends were turned in forming loops obstructed at both ends. Intestinal continuity was re-established by end-to-end or lateral anastomosis. The obstructed loop may be regarded as a form of strangulation obstruction because of the interference with the vascular supply as a result of the greatly increased intra-intestinal pressure. The intestinal anastomosis obviated the confusing factor of an additional simple obstruction which was found difficult of evaluation in the interpretation of the findings in a previous study even though lead shot were attached to the strangulated segment.

These dogs lived from 21 hours to 13 days (average 3.5 days), and post mortem examination revealed discoloration and distention of the obstructed loop, and peritonitis. Perforation of the loop was the cause of the peritonitis in one-third of the cases.

In 3 dogs the superior mesenteric vein was ligated. Two of these died within 4.5 hours but one young pup survived and appeared to be in good health when he was sacrificed after 13 days. Post mor-

[1] Wangensteen, O. H., and Lynch, F. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 674.

[2] Goehl, R. O., Lynch, F. W., Borman, C., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 952.

tem examination revealed no definite distention or discoloration of the bowel. Adequate collateral circulation had been quickly established to permit of return of the portal blood by other routes.

Experimental peritonitis was produced in 6 dogs by introducing liver, liver anaerobes or bile into the peritoneal cavity and by hepatic artery ligation. Death occurred in 13 to 48 hours (average 19 hours).

Results. X-ray films of the dogs with obstructed loops usually revealed some evidence of gas in the obstructed loop at the end of 8 or 10 hours, but the appearance of definite gaseous distention was an inconstant finding, occurring as early as 8 hours but in some cases not being definite after more than 100 hours. Fluid levels were demonstrable in some of the loops.

After mesenteric vein ligation, X-rays were negative in the 2 dogs dying early and in the animal that recovered gaseous distention was variable, being seen best at 23 hours, 72 hours, and 5 days.

Gaseous shadows were observed early (1 to 11.5 hrs.) in 4 cases of peritonitis but could not be satisfactorily demonstrated in 2 dogs dying at 13 and 20 hours.

From the experiments one may conclude that although X-ray evidence of gaseous distention is an early and valuable sign of simple intestinal obstruction it is distinctly of less value in strangulation obstruction or in stasis due to mesenteric vein occlusion. Gas shadows are also observed early as a rule in peritonitis.

5306

Technique of Spinal Anesthesia in the Dog.

HAROLD DVORAK AND M. H. MANSON. (Introduced by O. H. Wangensteen.)

From the Department of Surgery, University of Minnesota.

Lumbar puncture of the spinal canal of the dog has been practiced in the Laboratory of Experimental Surgery for the administration of regional anesthesia as well as during the course of other experiments. In the literature, however, it is generally conceded to be virtually impossible to perform successful lumbar puncture upon the dog.[1] We are describing the technique of the method in the hope that it may be of value to others. Out of 45 successive dogs upon which lumbar puncture has been attempted, 37 were suc-

[1] Ochsner, A., Gage, I. M., Cutting, R. A., *Arch. Surg.*, 1930, **20**, 802.

cessful; the failures were obtained in the earlier attempts when the technique was being elaborated.

The dog has 7 lumbar vertebrae. The spinous processes of these vertebrae in contrast to the human point cephalad. The vertebrae and their processes are closely united in the living animal, especially in the mid-line, and it is virtually impossible to pass a spinal puncture needle between them at this point, except between the last lumbar vertebra and the sacrum. Injection of anesthetics at this point does not ordinarily allow for diffusion to a level sufficiently high to induce true spinal anesthesia, and usually results only in sacral anesthesia. Laterally, however, there is a very definite interspinous aperture between the superior and inferior surfaces of any 2 adjacent lumbar vertebrae. Upon arching the spine of the animal, the aperture attains a diameter of about 2 to 3 mm., is about 2 to 3 mm. deep and connects directly with the dura. The axis of this canal in the sagittal plane is about 10 degrees with the frontal plane in the upper lumbar region, and about 45 degrees in the lower lumbar region. In the frontal plane, the axis of this canal is about 5 degrees with the sagittal plane.

In carrying out the procedure, morphine or some hypnotic such as sodium barbital, may be administered to make the animal lie more quietly. The dog is placed on his side with his hind legs tied closely to his fore legs, the head being tied closely to these.

In the performance of spinal puncture it is usually a good plan to aim the point and barrel of the needle in the direction of the spinal aperture, much as one shoots with a gun at a target. Because of the slight variations in the position and direction of this canal in the various levels of the lumbar spine, and the individual variations in different dogs, however, it has been found most convenient to follow closely the lateral surface of the spinous process down to the posterior arch; then with a slight probing motion the tip of the needle is directed downward, forward and medialward until it strikes the non-resisting tissues of the aperture. Spinal fluid is usually obtained at a distance of about 3 cm. from the tip of the spinous process.

The position of the needle in this aperture after trial puncture at post mortem has been carefully checked by numerous dissections of dogs' spines. The rate of flow of spinal fluid varies considerably in individual animals, ranging from a presentation of the fluid in the hilt of the needle, alternately receding with respirations, to an adequate flow of 20 to 25 drops a minute. As in spinal punctures on the human where no fluid is obtained, the flow may frequently

be started by slight manipulation of the needle, either rotatory or by slight withdrawal. A definite flow of spinal fluid was obtained in 34 instances of 37 successful punctures.

The injection of an anesthetic agent (novocaine) into the spinal canal was practiced in 16 of the 37 lumbar punctures, and was invariably successful, anesthesia to a high level being obtained. Two hundred milligrams of novocaine were used in large dogs, and 100 mg. in small dogs. Criteria for a high anesthesia were insensibility to pain to the intercostal angle (operations lasting two and a half hours, such as gastro-enterostomy and jejunal anastomosis, have been done), flaccid paralysis of the hind legs, relaxation of anal

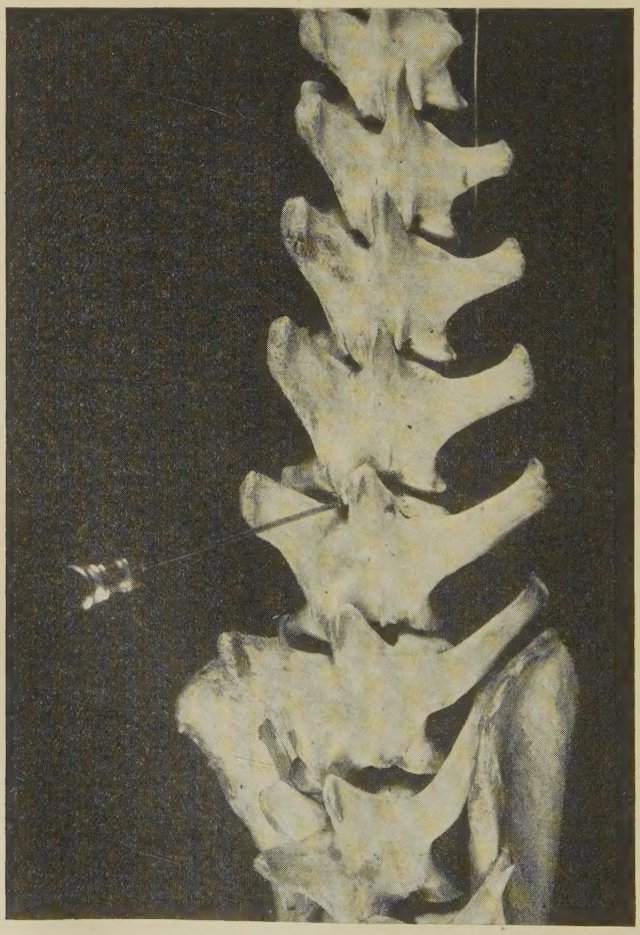

FIG. 1.

Lateral view of lumbar spine. Needle in space between 3-4 L vertebrae.

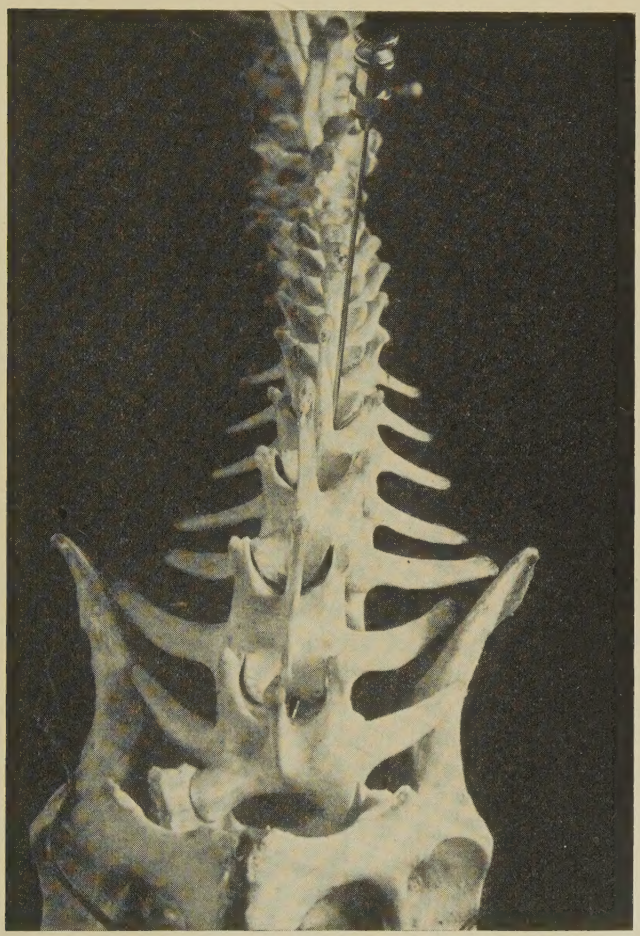

FIG. 2.
Spine from behind and above.

and urinary sphincters, increased intestinal tone, and characteristic immediate drop in blood pressure as evidenced by kymographic tracings.

5307

Finger Blood Method for Micro-Kjeldahl Non-Protein Nitrogen.

THOMAS L. CANNIFF. (Introduced by J. F. McClendon.)

From the Physiological Chemistry Laboratory, University of Minnesota.

Into a centrifuge tube, pipette 10 cc. of Folin's micro-tungstic acid solution. Make a reasonably deep wound in the finger to be assured of an adequately free and natural flow of blood, as squeezing appears to contaminate the sample. Using an accurate, capillary-bore pipette, draw up 0.1 cc. of blood. Carefully discharge the blood at the bottom of the centrifuge tube, and rinse the pipette with the clear supernatant solution; mix thoroughly. Allow to stand 2 or 3 minutes, and centrifuge. Decant the clear solution and apply a 4% correction for solution in the precipitate (or use a 5 cc. aliquot) and conduct a micro-Kjeldahl digestion. Collect the ammonia by distilling into 5 cc. of 0.1 *N* HCl. Fill the receiver tube containing the HCl up to the 25 cc. mark with distilled water, and add 5 cc. of Nessler's solution drop by drop while stirring. Add 5 cc. of the Nessler's solution to 25 cc. of the standard ammonium sulfate solution which should contain 0.1 mg. of nitrogen. Nesslerize the unknown and the standard simultaneously and compare in the colorimeter within 10 minutes.

TABLE I.

Blood from 1 fasting subject	Mg. per 100 cc. blood		
	Det. 1	Det. 2	Average
Venous blood	32	30	31 mg.
" "	33	30	31.5
Finger blood (decantation)	34.7	34.3	34.5
" " "	35.9	35.5	35.7
" " "	36.4	36.1	36.25
" " "	35.5	35.3	35.4
" " "	35.8	35.2	35.5
Finger blood (5 cc. aliquot)	40	37	38.5
" " "	38	38.1	38.05
" " "	36.8	39.3	38.05
" " "	36.8	37	36.9
" " "	44	47.2	45.6

The fasting level on finger blood was always found to run higher than that on blood from a veni-puncture, and the difference appears to be fairly constant.